The Journal of Laboratory and Clinical Medicine

VOL. VII ST. LOUIS, JANUARY, 1922 No. 4

ORIGINAL ARTICLES

BASAL METABOLISM DETERMINATION AND ITS TECHNICAL DIFFICULTIES*

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IN discussing the technical difficulties encountered in determining the metabolic rate, it is obviously impossible to enumerate the many possible sources of such errors inherent in the various methods and devices now in use for making metabolic rate determinations.

There are, however, a few basic principles common to all these methods and devices, a thorough understanding of which is absolutely essential to any degree of success in their manipulation and use. There is also at the present time a most urgent need for a better understanding of these principles, failure to observe which has led to more doubt in the minds of clinicians today, concerning the real value of metabolism studies in disease, than the sum of all the other confusing sources of variations combined.

Clinicians everywhere are asking the question: In what diseases other than thyroid and pituitary abnormalities may the basal metabolism test be useful? while equally great numbers of clinicians everywhere are failing to make thorough application of the test in these conditions, in which it is known to be the most useful diagnostic aid in their possession. This paper is, therefore, a plea to the clinician to use with better skill that which is already known to be genuinely useful.

In the process of determining the metabolic rate by this or that method, the final result is obviously and always the algebraic sum of the plus and minus errors from at least three widely different sources, namely: (1) the test subject, who though in normal health, may have a metabolic rate varying several per cent from his expected rate, and who may cooperate irregularly, or not at all, in the performance of the test; (2) the technician, who must execute many details and observations besides those pertaining directly to the manipulation of

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the instrument; and (3) the apparatus itself, which may introduce error, perhaps consistent, perhaps variable, because of certain of its mechanical defects unknown to the operator.

If the errors of various technicians making metabolic rate determinations were listed under these three headings according to who or what caused the error, it would be found, that (1) the test subject, (2) the technician, and (3) the apparatus, share quite unequally as causes of error. In this connection, it is one of the unexplainable features of the beginner's attitude of mind that when errors occur, he invariably declares that his apparatus is not accurate. The responsibility for all errors, regardless of their real source, is invariably laid to the fault of the apparatus. As a matter of fact, the apparatus is hardly ever the cause of error.

If the final result of a given test proves to be obviously incorrect, as the result, for example, of an air-leak in the apparatus, it is the fault of the technician for not having examined the apparatus for air-leaks at the beginning and end of every test in accordance with one of its fundamental requirements. On the other hand, if the rate is found to be too irregular in "check" determinations on a given subject, as the result of the subject's failure to lie perfectly quiet during the test, it is the technician's responsibility to require the subject to conform to certain fundamental rules, or else refuse to make the test.

Furthermore, if the technician's failure to observe these fundamental requirements is due to his lack of understanding of these sources of errors, it is again his fault for not having learned these essentials before assuming such responsibility.

In the Spring of 1920, the author had devised and perfected a portable device for measuring the rate of metabolism. This apparatus, in its separate parts, represented such wide departure from those of other devices then in use for this same purpose, that it was considered desirable, if not essential, to make comparison of its results with those of other devices then in use in the various nearby hospitals and laboratories. It was in this connection that the present writer was obliged to depend on the knowledge and technical skill of the various operators of these instruments with which the comparisons were to be made.

Of the six comparisons made, apparatus No. 1 showed an unmistakably low reading of –17% on the normal test subject, due, it was finally discovered to a previous exhaustion of the CO_2 absorbent; apparatus No. 2 gave a +21% reading on the same normal subject, due, it was found after an entire morning's delay, to a leakage of air from a large rubber-hose connection; apparatus No. 3 gave a –12% reading, due it was discovered after three days of "tinkering" with the apparatus itself, to an incorrect factor which had up to this time been used in the calculations of the gas volumes; apparatus No. 4 showed so many defects that the experiment was abandoned after three readings showing +72, +90, and +20 per cent. A fifth attempt was made by returning, then, to apparatus No. 1. This time the comparison was fairly successful, showing only a 7 per cent difference in the readings of the two instruments, but even here the reading from apparatus No. 1 was again incorrect because the technician had neglected to interpolate to the second decimal place for the body area, which

she admitted had been her regular custom. A sixth attempt was then made with apparatus No. 4, but a wide disagreement was found again because the technician according to his regular custom, stopped the electric motor immediately after disconnecting the subject from the breathing circuit, which obviously left a considerable volume of unabsorbed CO_2 in the spirometer and breathing circuit.

Lest it be thought that this was a most unusual series of mishaps in making these comparisons, I feel impelled to admit that subsequently it has been my experience to find this deplorable state of affairs throughout all parts of the country. And any technician or clinician who feels that his own results are an exception to this, should see to his own technic in securing *normal readings on known normal subjects* before offering challenge to the proposition.

Usually if the reading on a suspected hyperthyroid subject falls by chance on the plus side of that patient's normal rate, the clinician accepts the result without question, because "it checks up clinically." That the subject may not be suffering from hyperthyroidism at all, or that the plus reading may be more or less "plus" than the rate really is, does not matter to him, so long as the results "check up clinically." With some, the source of error is from the calculations; with others it is from misreading the stop watch, or lack of a stop-watch, or the barometer, or lack of a barometer; with still others it is lack of information concerning the patient's preparation, and so on.

When told of the unsatisfactory results of other comparative tests, each technician, in turn, invariably hastens to assure one of the wonderful accuracy of his instrument. Others may be in doubt about their instruments, but he knows his apparatus is accurate. Does he make tests on subjects known to be normal? Why certainly not—normal subjects are not interesting. Does he run duplicate tests on his patients? No, duplicate tests would only give the same result, and besides they take too much time. Then, how does he know that his apparatus is accurate? Simply because the results always "check up clinically!"

This reassuring conversation is almost invariably followed by the usual disappointing result, namely, duplicate readings on a normal subject which are ridiculously at variance with each other, and which also disagree with what is known to be the normal rate. His failure this time to get his tests to "check up clinically" is registered as a definite depression in his enthusiasm, but not to be outdone, he still assures one that this is the first time the apparatus has been out of order, laying the blame again, in characteristic fashion, on the unoffending apparatus.

Unfortunately too many technicians merely skim through the reading of instructions and therefore have only a very superficial grasp of the underlying principles of apparatus and methods committed to their care. Only after a technician has had much experience in metabolism studies, will he appreciate what a really exacting task it is, this measuring the rate of metabolism. Any device used for this purpose, however simple and mechanically perfect, requires careful watching, as does any other exacting quantitative test, and the technician who gets into difficulty in making such a test is invariably one who has read his instructions hurriedly, and understands them superficially, or not at all.

Therefore, it is absolutely essential that the technician take time to read instructions carefully, and to read them through to the end before attempting to perform the test. Obviously each important point cannot be emphasized separately because every step in the technic is important, and no matter how perfectly the technician performs most of the test, the conclusiveness of the final result is no stronger than the weakest point in the rest of his technic since these are also links in the same chain, which produced that final result.

One should study the apparatus itself, in connection with the instructions, understand the purpose and working of each part, and follow out the reasons and principles involved in the whole procedure. They are merely elementary facts of science, long ago familiar to the technician, and their relations are so perfectly obvious when once connected as logical means to our aim, namely, to measure the rate of oxygen consumption. Only in this way will the clinician be in a position to interpret unexpected variations in the results, and to disclose and eliminate those hidden causes of error which are invariably encountered in any quantitative measurement.

Since (1) the test subject, (2) the technician, and (3) the apparatus, are severally responsible for the errors encountered in these tests, it is only common sense for the beginner to start with one of these factors as a known and relatively fixed quantity, namely, a test-subject in normal health, who will cooperate in maintaining for him at least relatively uniform basal metabolism conditions for practicing the test. In this way the other two sources of error may then be reduced to known quantities, and, in turn, eliminated.

Most every technician feels that, at least for him, this preliminary training is an unnecessary waste of time. After the first few attempts with the test one will readily understand why this try-out method is recommended for the beginner. On over one hundred different occasions the present writer has witnessed the beginning technic of over one hundred clinicians or their technicians. Out of this number, not one of them, during their first attempts, came anywhere near the proper handling of the various types of apparatus used for this purpose, and showed even less knowledge as to how the patient should be managed during the test. The test itself is usually so simple, and some of the foolish blunderings of otherwise intelligent technicians are so inexcusable, and their mistakes in diagnosis of such far-reaching consequence, that it has been a source of constant wonder that suits for malpractice are not the rule rather than the exception through the abuse of this most valuable diagnostic aid.

Another peculiar feature of the beginner's attitude of mind with reference to apparatus used for determining the metabolic rate, is that invariably, when left to follow his own desires, he turns at once to pathologic cases to "try out the apparatus." Such an "accuracy test" would be even more illogical than testing the accuracy of a clinical thermometer on a patient with an unknown rise in temperature.

Practically every error in technic which the beginner is likely to make is such as would lead to unusually wide variations in the final reading of the test and therefore to gross error in diagnosis. Thus, in spite of repeated warning, one favorite blunder of the novice is to read the time, e.g., 2 minutes and 55 sec-

onds, from the stop watch as 3 minutes and 55 seconds. Warning him of this is useless. He thinks he should at least know how to read a stop-watch.

The reason for the frequency of this error is that the minute-hand of the watch stands so near to the succeeding minute mark when the second-hand is approaching the last few seconds of the preceding minute. To ask a technician to avoid this particular error is an obvious reflection on his intelligence. When, in spite of warning and explanation, however, he finally is caught in the error, he usually flushes up a bit and passes it off, as though it amounted to nothing anyway, not knowing that it amounts to about a 25 per cent error in the final calculation of the metabolic rate.

For example, on one occasion the technician reported a +8 per cent reading, on a suspected hyperthyroid patient. This rate being within the normal limits, the patient was dismissed as a "neuro." The physician four months later became convinced that some error in the test had been made, and ordered the technician to make a second test. This time, however, the technician who had meanwhile learned to avoid the above source of error, found the rate to be +58 per cent. This unexpected increase and the record sheet of the previous test led to the discovery that, because of a reading of 3 minutes and 52 seconds, the rate as determined two months ago should have been reported as +35 per cent instead of +8 per cent. Now, however, the patient was so much worse, that radical operation, although advised against, when performed two weeks later, eventuated fatally. One can say nothing less than that failure to observe a specified point in the technic had allowed the golden moment for successful operation to slip by.

On another occasion, a clinician was demonstrating the metabolism test to a group of visiting clinicians. He had never made the test before, and the apparatus to be used for this purpose had just been received. In characteristic fashion, he had turned at once to pathologic conditions, and had gone to much trouble to have a hyperthyroid patient serve as his subject. The demonstration had been a failure after several attempts, and it was at this point that the clinician called me by telephone to ask how so and so's method was supposed to affect the patient.

Half an hour later, when I had joined the other visitors, the test was tried once more. When the test began, the patient was comfortable, breathing normally for the first 30 or 40 seconds, but after about one minute, the patient drew up her knees, rolled the eyes upward, changed to a deep sonorous type of respiration, the face became deeply cyanotic, the jaw relaxed, and fortunately for her, allowed the mouth-piece to drop from the mouth.

Gas anesthesia!

Not by the wildest flight of imagination could the investigator, who designed this new method, have conjured up the possibility of so huge a blunder, or anticipated a warning to those who used it, not to give gas anesthesia when doing metabolism determinations. A tank of gas, supposedly oxygen, but labeled "Nitrous Oxide" stood nearby as mute witness to the clinician's "oversight."

Many other equally unfortunate instances could be cited to show how absolutely essential it is to *have the technician practice with known normal persons,*

whose metabolic rates are known, within reasonably narrow limits, and whose management during the test is relatively simple as compared with the tactful and varied procedures necessary with the highly excitable hyperthyroid patient or the temperamental neurotic. To demonstrate his ability to make a reading sufficiently dependable for clinical use, it is only common sense to require that he begin with at least one known quantity, namely, test cases of known metabolic rate.

One should not imagine, however, that the values given in the table of so-called normal rates, are absolute values. A normal person may vary above or below these values (commonly 10 per cent, rarely 20 per cent) and still be regarded as in good health. These rates should be called averages rather than normals. Most of the readings, however, if properly made, will come within a few per cent of these values, but one should not, as some technicians do, become discouraged if the first normal subject tested should chance to be one of those rare cases whose rate varies regularly several per cent above or below the expected rate. One should simply continue the tests on still other normal persons, until the cause of uncertainty is discovered.

Moreover, when the technic is finally mastered, and the technician has become thoroughly acquainted with the features which require constant watching, he should, in the midst of his many tests on pathologic subjects, frequently return to known normal subjects to reassure himself that some undetected mechanical defect in the instrument has not developed recently, and thus introduced an unexpected source of error. These insidious sources of inaccuracy are far more numerous than commonly known, and the only method by which they may be detected, and which is always available to the technician, is to return again to fundamentals and require the instrument to give normal readings on known normal persons.

That the skill and knowledge of the technician must be adequate to the task of producing a normal reading on a normal subject is so obvious as to require no argument, but the difficulty seems to be that this amount of skill is taken for granted, and the clinician wants a short cut to direct results with his cases showing real pathology.

Another limitation of the technician's skill no less important than his ability to secure normal readings on normal subjects, is his ability to secure uniform results in successive tests on the same subject under the same conditions. Is his technic so standardized that two or more determinations on the same subject tell the same story?

Here, again, such an obvious situation is taken for granted, namely, that the same causes must produce the same results, and time is too valuable to spend in useless, uninteresting repetition.

One clinician, for example, when asked how his readings on the same subject agree with each other, replied that he never made duplicate determinations since any one test was always accurate to at least one one-hundredth of one per cent, and that this was close enough for his purpose, without a duplicate test! This technician did not know that the average limits of accuracy of any method

devised so far, show at least 2 per cent variation in its own readings, and that the method used by him is the most variable of them all.

Then, on the other hand, the technician who has been told the limits of accuracy of such and such an instrument or method, takes it for granted that this accuracy is an inherent part of the device itself.

Devices may differ in the number of manipulations necessary to reach the end result, and, in this way, in the number of chances for possible errors through such manipulations, but they do not differ in their absolute dependence upon a thorough knowledge of the technic required for their operation.

Regardless of the method or device employed, the rate of metabolism is stated in terms of calories, these calories in turn being determined in terms of the amount of oxygen used by the subject. Therefore, *the brunt of the calculation falls entirely upon the accuracy with which the oxygen volume is measured.*

Now, since gases, which must be passed and repassed through tubes, spirometers, rotary-blowers, face-masks, mouth-pieces, gas-cocks, and what not are notoriously subject to rapid losses through the most minute points of escape, the amount of escape being the amount of error in calories, the latter may very quickly become considerable.

Since these points of escape may develop at any time between tests, or even during the test, a single determination may be seriously in error without obvious cause unless the gas-tightness of the apparatus be proved before and after the test, as a routine part of the technic. With certain forms of apparatus a leak will not develop after the apparatus is once closed ready for the test proper, but with other forms this mishap is common, and it is clearly the technician's responsibility to be constantly on guard against such unexpected sources of error.

By using the duplicate test as a regular part of the routine, these and other irregular causes of error, such as face-mask or mouth-piece leaks, errors in calculation, misreading the stop-watch, and so on, are also detected, and many an embarrassment in diagnosis spared the clinician.

SUMMARY

1. The technic of the operator must be proved adequate to the task of securing normal readings on known normal subjects. One need not quibble about this. It may be difficult to secure the service of a normal person for a subject, it may take much of one's valuable time to make these uninteresting tests, but *the clinician positively cannot rely on his results with suspected pathologic cases until this fundamental principle has been observed.* The reason this principle needs such repetition and emphasis is that every clinician, without exception, imagines that the apparatus is accurate if it will give a plus reading on a known hyperthyroid patient. It does not occur to him that such a test is absolutely no proof of accuracy, since the apparatus may be very inaccurate and still give the desired plus reading. In other words, the reading may be any amount of per cent plus and the clinician still be in no position to dispute with the instrument whether or not this is the correct plus percentage.

2. Having learned the technic on normal subjects the tests may subsequently, in the routine of business, become a long sequence of determinations on only pathological cases, during which time some unknown mechanical defect may have introduced an undetected source of error. This requires that the clinician return to fundamentals again, and eliminate the possibility of these sources of error by securing normal readings again on subjects known to be normal, *not depending for this on occasional subjects sent in as patients which may or may not be normal.*

3. If any quantitative determination is unsafe without a duplicate determination, or appropriate controls, which serve the same purpose, basal metabolism determinations are doubly unsafe, because of the great complexity of details in the technic and the far-reaching consequence of their errors. Common sense will sanction the statement that this applies to any device or method used for this purpose, regardless of who or what institution vouches for its accuracy, and regardless of the amount of time required for duplicate tests. A method which requires so much time for one test as to make the two tests impracticable, is unfortunate in requiring so much time, but is not thereby made an exception to the fundamental rule that *any method or device, to be dependable, requires a duplicate test for a control.*

Recently a physician asked to have two check determinations made on himself, by what has been widely reported as the most accurate method now in use, and by a technician who had made determinations then numbering into the hundreds, but who had always omitted the check test for lack of time, "for such unnecessary labor." The two readings were −21 per cent and −7 per cent, respectively. Somewhat embarrassed by this disagreement, the technician agreed to make two more determinations on the following day. This time the readings were +4 per cent and −4 per cent, respectively. This physician was so genuinely disappointed with the results that his honest cooperation during the tests cannot be questioned. Furthermore, he subsequently went over the results of the gas analysis and calculations with the technician to assure himself that the wide variations were not from these sources. According to report, such wide variations on the same subject do not occur, but in practice they do, and in the hands of the unskilled, they are even greater than these. When they will occur, can never be known beforehand. Why they occur, is another story.

THE INFLUENCE ON SPORE FORMATION OF SEALING BACTERIAL CULTURES*

By Laura Florence, Ph.D.

DURING a study of the pathogenesis of *Bacillus abortus* Fabyan (1912), continuing the work of Smith and Fabyan (1912), stated that the chief problem in the cultivation of the organism was to obtain successful cultures from the tissues. The method followed was that of Nowak with slight modifications, but later it was found that by merely sealing the culture tube with sealing wax, and incubating it longer in some cases, satisfactory results were obtained. Since then the latter method has replaced all others in this laboratory in the cultivation of *Bacillus abortus*. Also, it has been the means of the discovery by Smith (1918, 1 and 2) of two new organisms, viz.: the *Vibrio fetus* of infectious abortion in cattle and *Bacillus actinoides* which may be representative of a hitherto unknown group of organisms capable of growth, at least at the start, only under such conditions. The question naturally followed, what is the general effect on culture growth of such a method? To discover this a series of experiments was begun during the winter of 1918-1919 and transfers of a number of stock cultures were grown in parallel series of sealed and unsealed cultures. The results showed the necessity of confining the observations to some well-defined biological process, and spore formation presented itself as the most suitable. The work was limited first to a study of some of the common sporebearers and finally to a more detailed study of the anthrax bacillus.

The organisms used in this investigation were *Bacillus cereus, Bacillus mesentericus fuscus, Bacillus mesentericus vulgatus* (2 strains,) *Bacillus Welchii, Bacillus anthracis,* and two unidentified bacilli, one isolated from the lung of a calf and designated *"Organism A,"* and one isolated from the liver of a guinea pig and designated *"Organism B." Organism A"* is in size and appearance not unlike *Bacillus mesentericus vulgatus.* It is actively motile and central spores appear within 24 hours. On solid and liquid media it grows as a wrinkled membrane. In bouillon a faint turbidity appears in 24 hours and gradually increases in density during the life of the culture. It grows on potato, liquefies gelatin, and slowly coagulates milk with an extrusion of whey on the 4th day. In the sugars there is no gas production and the reaction is moderately acid. *"Organism B"* is a slender rod with rounded ends. It has a moderate motility and forms small terminal spores. In deep agar shake cultures, small subcircular colonies with an irregular surface appear within 24 hours and, as they grow, they become rhizoid in form. Numerous gas bubbles are formed.

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Received for publication, July 7, 1921.

To determine the relation of these organisms to oxygen, cultures were grown in fermentation tubes of dextrose bouillon after the method of Smith. In 1893 he had explained the use of the fermentation tube for such purposes and two years later (1895)) emphasized the relation between sugar and anaërobiosis. The results of the test grouped the organisms as follows:

1. Obligatory aërobes	Bacillus mesentericus fuscus Bacillus mesentericus vulgatus (2 strains)
2. Facultative anaërobes	Bacillus anthracis Bacillus cereus Organism A
3. Obligatory anaërobes	Bacillus Welchii Organism B.

A uniform method of closing the culture tubes was used. All agar slants were closed immediately after inoculation with paraffin-dipped cotton stoppers cut off level with the top of the tube and then pushed down approximately one-sixteenth of an inch below the top. The mouth of the tube was flamed until thoroughly hot, when a small amount of sealing wax was placed over the stopper. This was absorbed by the stopper and, when the tube had cooled, the space above the stopper was filled with sealing wax, care being taken to leave no air bubbles. Cultures grown in bouillon and on potato were closed in the same way as the agar slants except that the stoppers were not dipped in paraffin.

Under such conditions the development of an organism is affected by a number of factors. As already pointed out, such an environment favors the growth of some organisms. Moreover it had been stated by Pasteur (1880) that hermetically sealing the necks of his flasks preserved the virulence of his cultures of the organism of chicken cholera over long periods of time. In spite of the exclusion of the air two sources of oxygen may still be available to the organisms. There is first that enclosed in the tube when sealed, and second that which may be contained in the media. It was found by Hesse (1893) that, during the growth of organisms in markedly alkaline media in air-tight tubes, a considerable quantity of the available oxygen was absorbed by the media, but in media of such moderate alkalinity as is usual in routine work the quantity of oxygen so absorbed was negligible. However, that a reduction in the oxygen tension does gradually take place in sealed tubes of media of a moderate alkalinity can be proved by adding a few drops of a sterile aqueous solution of methylene blue to the tubes at the time of sealing. Reduction phenomena coincident with bacterial growth have long been known, but Spina (1887), alone of the earlier workers, has described its occurrence in sterile media—bouillon and gelatin—in hermetically sealed tubes. Some nine years later Smith (1896), while using the fermentation tube in his study of the reducing action of bacteria, also found that sterile culture media exhibit reducing properties. In these two cases the reduction takes place in what may be described as an air-free environment with consequent rapid loss of color. If, on the other hand, the colored medium is contained in ordinary culture tubes closed with sealing wax, there will elapse, before the color change takes place, a variable period of time.

The experiment was carried out with bouillon and agar; each tube contained 6 c.c. of medium and the agar was slanted. The medium was placed in a water-bath and after reaching boiling point was allowed to boil from 10 to 15 minutes. It was cooled quickly to 45 to 47°C. and definite quantities of a 0.1 per cent sterile aqueous solution of methylene blue added. One-half of the tubes were cooled before sealing and the remainder were sealed immediately on taking from the water-bath. The tops of the latter were thoroughly heated in the flame, a tightly fitting circle of sheet asbestos was placed over the cotton, and they were then sealed as quickly as possible. The asbestos prevented the sealed plug from being drawn into the tube as it cooled. The results given in Table I show not only that the decoloration took place slowly but also that the time taken was not uniform in the different tubes.

TABLE I

MEDIA	QUANTITY OF 0.1 PER CENT METHYLENE BLUE	NO. OF DAYS FOR COMPLETE DECOLORATION IN INCUBATOR		NO. OF DAYS FOR COMPLETE DECOLORATION AT ROOM TEMPERATURE	
		SEALED HOT	SEALED COLD	SEALED HOT	SEALED COLD
	c.c.				
Agar	½	37	40	33	–
		40		30	33
		69	69		
	¼	18	–	22	33
		17	–	20	–
	⅙	12	54	17	35
	⅐	13	20	17	20
Bouillon	½	64	–	–	33
	¼	14	–	–	26
	⅙	–	13	19	–

Tubes sealed hot become quite decolorized a little more rapidly than those allowed to cool before being sealed. The rate of the reduction was also moderately influenced by the temperature at which the tubes were kept, and was to some extent proportional to the quantity of coloring matter introduced.

In the study of the common spore-bearers, bouillon and agar were the media used. If transfers were made from cultures containing spores, the majority of the spores carried over did not germinate in the sealed tubes, so it was necessary to make the transfers from cultures free of spores. To obtain these two methods were tried; first, making transfers from cultures grown 6 to 8 hours, and second, heating freshly inoculated cultures containing spores above the thermal death point of the vegetative forms and incubating them for a number of hours before sealing to allow the surviving spores to germinate. The former procedure was the more satisfactory and was used almost entirely throughout the experiments.

EFFECT OF SEALING ON MACROSCOPIC GROWTH

In order to determine the effect of sealing on macroscopic growth the aërobes and facultative anaërobes, with the exception of the anthrax bacillus, were first grown in bouillon cultures, when the following results were obtained:

UNSEALED CULTURES

All organisms grew well.

SEALED CULTURES

The aërobes showed a very moderate growth in 24 hours which had not visibly increased at 48 hours. After 5 days the culture of *B. mesentericus fuscus* appeared like sterile bouillon, while the others showed traces of a thin surface membrane which later disappeared.

The facultative anaërobes grew as well as in unsealed tubes for 48 hours, when macroscopic growth ended. After 9 days the growth began to disappear and after 23 days only a small amount of sediment remained to distinguish the cultures from sterile bouillon.

In films made from sealed bouillon cultures incubated 37 days without having been unsealed, few organisms were found, vegetative forms and spores being present in approximately equal numbers. When the cultures were unsealed and incubated, growth appeared in all before 48 hours. In films made from sealed agar cultures incubated 75 days without having been unsealed, vegetative forms and spores were again found in approximately equal numbers. When the agar cultures were sealed and incubated, growth appeared in all after 3 days.

EFFECT OF SEALING ON SPORE FORMATION IN AEROBES AND FACULTATIVE ANAEROBES

In determining the time of appearance of spores parallel sets of cultures on agar were grown in all cases, some of which were examined daily, and others at intervals of 2, 4, and 6 days. Of each of the aërobes and facultative anaërobes a series of thirty to forty inoculations was made at one time, to be opened on successive days, but the results did not differ materially from those got from cultures repeatedly opened for examination and resealed. By series of cultures is to be understood all the inoculations of an organism made at one time. A study of these series showed the following results:

UNSEALED CULTURES

All organisms grew well producing spores within 24 hours.

SEALED CULTURES

In the first series of aërobes spores appeared after 27 to 30 days' incubation, and in a second series, inoculated from the first, after 32 to 36 days' incubation.

In the first series of facultative anaërobes, spores appeared after 25 to 35 days' incubation in "*Organism A*," and after 19 to 26 days' incubation in *Bacillus cereus*. In a second and successive series to the sixth, spores appeared after 30 to 35 days' incubation.

In rate of growth and cessation of growth agar cultures resembled bouillon, but on the former the organisms died more slowly. When spores first became visible they were present in very small numbers and in most cases were still within the vegetative forms. The change from vegetative forms to spores took place very gradually and in cultures that had been incubated 60 to 75 days, spores within vegetative forms were found. Some of these organisms may have died in that condition, but such cultures, if unsealed and incubated, showed new growth in 2 to 3 days.

Parallel series grown in tubes sealed 1, 2 and 3 weeks before inoculation and kept in the refrigerator, at room temperature, and in the incubator, gave results corresponding to those obtained from cultures grown in tubes sealed first at the time of inoculation. This might not be the case if it were possible to inoculate the tubes without exposing them to the atmosphere.

Parallel series grown at room temperature and in the incubator did not differ appreciably in growth or in the results obtained.

After five days for the aërobes and 9 days for the facultative anaërobes the number of the organisms in hanging drops and films began to diminish. That the majority died without forming spores was suggested by the many nonmotile forms present in hanging drops and the many involution forms in films.

EFFECT OF SEALING ON SPORE FORMATION IN OBLIGATORY ANAEROBES

In the case of the two obligatory anaërobes no retarding of spore formation occurred, and the presence of spores at all stages of growth was confirmed by means of heat tests. Certain facts seemed to indicate, however, that the conditions under investigation were not highly favorable to these organisms. In agar slants, to the condensation water of which small pieces of sterile guinea pig tissue had been added, no better growth was obtained in sealed than in unsealed tubes. In both cases the growth appeared rather slowly and a number of such transfers were negative. Transfers made to agar slants without tissue and sealed rarely showed growth and, if left unsealed, no growth appeared. In these some of the vegative forms carried over at inoculation may have formed spores, but in many cases they appeared to die without undergoing any change, as they were found in films and hanging drops of cultures which gave negative results, when transfers were made from them to agar shake cultures.

Bacillus Welchii grew somewhat more readily than *Organism B*, growth appearing within 24 hours in the former and usually within 48 hours in the latter but in a few cases not for several days. The earliest indication of activity of the organisms was the formation of gas between the curved surface of the agar slant and the glass. The bubble increased in size generally for a period of 2 days, when the gas escaped wholly or in part from behind the agar. The formation of gas was followed by a slow growth of the organism between the medium and the glass. *Organism B* grew in a thin diffuse film and *Bacillus Welchii* in well-defined colonies. In sealed agar slants containing guinea pig tissue the organisms retained their vitality for a considerable time, and transfers to deep agar tubes from cultures which had been incubated upwards of 150 days gave positive results. Films stained with methylene blue and hanging drops showed vegetative forms in cultures 144 days old. The presence of numbers of dead vegetative forms in old cultures would indicate that the conditions were not altogether suitable for spore formation, though a limited number of spores were always present.

THE ANTHRAX BACILLUS

The results obtained when the anthrax bacillus was grown in sealed culture tubes proved to be so variable that a number of experiments were carried on with this organism alone. Two strains were used but no difference was detected in their reaction to the conditions of growth. A number of publications dealing with spore formation in anthrax appeared at the beginning of the present century. These will not be discussed here, because the writers

were experimenting with conditions of complete anaërobiosis and moreover, they have already been reviewed by Sobernheim (1913).

In a series of eight transfers of the anthrax bacillus made from a spore-free culture and grown on sealed agar slants, spores were not found in seven cases until after the 27th day. The examination of films and hanging drops of the cultures showed that the majority of the vegetative forms did not retain their normal outline as was the case with the other spore-bearers studied, but gradually came to resemble single cocci and chains of strepto-cocci. This experiment was repeated four times and in no case were the definite results of the first experiment obtained.

The organism was grown in sealed culture tubes and flasks of different sizes to discover the bearing of the quantity of enclosed atmospheric air on the time of appearance of spores. Culture tubes of the usual diameter but of three different lengths, viz. 12 cm,. 15.5 to 16 cm., and 20.2 cm., were used. No appreciable difference was found between the results obtained with the first and second of these, but the time was considerably shortened when the third length was used. In the former, spores were first found after 27 to 36 days but in two cases none were found after 64 days, while in all the latter, spores appeared at the 10th day. That the organisms in the 64-day culture were still alive was proved by the growth of a transfer made from it before the heat test for spores was made. Cultures were also grown on agar in Erlenmeyer flasks of 100 c.c. and 250 c.c. volume and in Blake bottles of 500 c.c. volume. In the two last, spores regularly appeared within 24 hours and in the first within 48 hours. Bouillon was substituted for agar in the 100 c.c. flasks, 10 c.c., 20 c.c., and 30 c.c. being put in each of three flasks, and in every case spores formed during the first 24 hours. These results showed that as the ratio between the surface of the medium and the volume of the air enclosed was increased, the time before the appearance of spores was decreased.

Since the anthrax bacillus grows well on potato and as a rule numerous spores are produced while the culture is still young, tubes of potato similar in size to the usual agar slant tubes were prepared in three different ways and two varieties of potato used. Sobernheim (1913) considered the variable chemical composition of potato, especially its reaction, to influence not only the growth of the organism but also spore formation. Five series of cultures were grown on potato. In the first three series the potato was prepared according to the usual laboratory method and washed for 2 hours in running water, in the fourth series three potatoes of the same variety were used and were washed in running water for less than 1 hour, and in the fifth series potatoes of two different varieties were prepared also with short washing. In every sealed culture of all five series spores were present at the end of 24 hours and were almost as numerous as in the parallel series of unsealed cultures. Growth in the unsealed tubes was characterized by the formation of a brownish pigment, while in the sealed tubes it was always a smooth glistening white. Special potato media were next prepared. A potato was peeled and cut up, boiled to a pulp in distilled water, strained through muslin and 1 per cent agar added to the extract (a). From others (b) the starch

was separated, and extracts made containing (c) the salts with the protein and (d) the salts without the coagulable protein. These preparations were each made up with 1.5 per cent agar. Parallel series of sealed and unsealed cultures were made on all these media. The organism grew well on the potato extract agar (a) forming a brown pigment in the unsealed tubes; it grew moderately well on the salts with protein extract agar (c), and on the salts extract agar (d), but it did not grow on the starch extract agar (b). The results coincided with those obtained from the usual potato cultures, and in all tubes, sealed and unsealed, in which growth occurred, spores formed within 24 hours after inoculation.

In order to discover whether the reaction of the media influenced spore formation in sealed cultures, acetic acid was added to three series of bouillon tubes whose resulting titratable acidity varied from 0.45 per cent to 2.45 per cent and pH from 7.8 to 5.8. Also to six series of agar slants normal sodium carbonate or acetic acid was added to produce titratable acidities varying from 0.1 per cent to 2.8 per cent. In both experiments the results were variable and showed that some important factor influencing spore formation had not been found. Growth on beef extract agar with titratable acidities corresponding approximately with those of the above agar series threw no further light on the point under investigation, but it was of interest that neither in unsealed nor sealed tubes having the two highest titratable acidities would the organism grow. The explanation was found to be lack of buffer substance and, when the buffer value of the media was increased by the addition of phosphates without changing the hydrogen-ion concentration, pH 6.8, the organisms grew equally well in the whole series.

When sugar-free fermented bouillon and agar were used, spores regularly appeared within 24 hours after inoculation in both sealed and unsealed cultures. The addition of a 0.1 per cent of a 0.2 per cent solution of dextrose to two parts of the bouillon slightly accelerated the rate of spore production.

The fact that on the special media prepared from potato the organism had grown moderately well on the salts extract agar (d) suggested that salts might be an important factor. 1 c.c. of $\frac{M}{5}$ solutions of the potassium phosphates were added to 6 c.c. portions of agar and the medium sterilized and slanted. In all cultures spores developed within 24 hours. The sodium phosphates were used in two experiments along with three different media. In the first experiment a constant pH 7.4 was maintained and 1 c.c. portions of seven different concentrations of the salt were added to 6 c.c. of media, while in the second experiment one salt concentration, $\frac{M}{2}$ was added to give five different hydrogen-ion concentrations, viz., pH 4 to pH 8. The three types of media used were (1) plain beef agar, (2) 1.5 per cent agar in distilled water, and (3) the salts extract of potato (d). All the cultures grew well on (1) showing that the salt had no toxic effect in the concentration used and numerous spores were produced in less than 24 hours. On the second medium (2) no growth occurred. In the presence of the salts from potato, medium (3), spores ap-

peared in both sealed and unsealed tubes within 24 hours, and the best growth was obtained on the media having a hydrogen-ion concentration of from pH 6 to pH 8. Poor growth was obtained on media with a hydrogen-ion concentration of pH 4 or to which a very low concentration of the phosphates had been added. In the latter case the organism seemed to grow slightly better in the sealed than in the unsealed tubes.

To potato extract medium (d) were also added (1) a 1 per cent solution of casein aminoids, (2) a 1 per cent solution of asparagin, and (3) an 0.2 per cent solution of dextrose. These solutions used alone or in a combination of casein and sugar and asparagin and sugar did not favor the growth of the organism, and spores were formed in less than 24 hours in all cultures. The addition of casein along with the sodium phosphates to the potato extract agar (d) influenced neither the growth of the organism nor the rate of spore production.

In the course of the above experiments repeated comparisons were made of films and hanging drops prepared from sealed cultures grown on ordinary agar with those prepared from sealed cultures grown on media to which salts had been added. The presence of salts was found to increase the rate of spore production and, since both potassium and sodium salts produced the same effect, it may be the regulation of the reaction by the salts which is an essential factor in controlling spore formation under partial oxygen tension.

MORPHOLOGY

The exclusion of a free supply of atmospheric air brought about a marked change in the morphology of the anthrax bacillus, while in the other forms studied, very little if any change appeared in the vegetative forms. The process of spore formation in anthrax was studied by Ruzicka (1910) and he used a special medium which retarded the growth of the organism. This condition affected the vitality of the bacilli and the first steps of the changes which he described are identical with those seen in cultures grown in sealed tubes. According to Ruzicka the contents of the individual organisms lost their homogeneous appearance and became markedly granular, while some of the granules reached a considerable size and appeared black and refractive, not unlike small pores. Similar forms were later occasionally seen by Sobernheim (1913) in unsealed cultures grown on potato. In sealed cultures we have usually found them in considerable numbers after 3 to 4 days' incubation and in hanging drops they resembled cocci. The protoplasm surrounding the granules disappeared leaving only the refractive parts and before these became quite detached from one another they resembled short chains of streptococci. That these were not ripe spores was proved by the facts that they stained readily with methylene blue and were not heat resistant. *Bacillus anthracis* is Gram positive, but these forms were Gram negative. Since their formation was identical with the first steps in spore formation as described by Ruzicka, they were probably spores whose complete development was prevented by lack of sufficient oxygen and might be called spore-like bodies. In hanging drops made from cultures which had been sealed for a number

of weeks without being opened, almost all the organisms were of this coccoid form and, if such cultures were left unsealed and incubated, in less than 24 hours these had reverted to normal vegetative forms. Also, in tubes which were being repeatedly opened for examination these spore-like bodies were less numerous, even in the oldest cultures, than in tubes which were seldom unsealed. In hanging drops they were at times seen clumped together in zoögleic forms and at times they showed an active Brownian movement. The former had been noted as characteristic of anthrax bacilli in the absence of oxygen by Wood in 1889. Attempts were made to transfer these spore-like bodies to agar slants which had been sealed when the original culture was made and incubated along with it, but their form was not constant and they gave rise always to typical vegetative forms. They cannot then be considered mutants such as Henri (1914) obtained by exposing cultures of anthrax to the ultraviolet ray.

SUMMARY

The reduction of methylene blue in culture tubes of sterile media closed with sealing wax proves that a reduction in oxygen tension can be obtained by this simple method. The moisture of the medium is also preserved over a long period.

Sealing cultures retarded the formation of spores and killed the majority of the vegetative forms of the aërobes and facultative anaërobes but not of the obligatory anaërobes. It affected the vegetative growth of obligatory aërobes more rapidly and intensely than that of facultative anaërobes. It did not apparently affect the growth of obligatory anaërobes as compared with that in unsealed culture tubes containing a small piece of sterile tissue.

Among the aërobes and facultative anaërobes spore formation was retarded a few days longer in the second sealed series inoculated from the first than in the first sealed series, after which no variation in the time of appearance of spores occurred in cultures carried through six successive series.

The results obtained with the anthrax bacillus differed from those of the other facultative anaërobes and showed no uniformity. Experiments with different salts appeared to increase the rate of spore production and the regulation of the reaction by the salts may be an important factor controlling spore formation under partial oxygen tension. The absence of a free supply of oxygen caused a temporary change in the form of the bacillus.

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SOME STUDIES ON THE ELIMINATION OF MOLDS FROM BUTTER*

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THE following paper is a summary of the work done for a large butter-making plant which was troubled with their butter going "off grade" during the interval between the time it was shipped and the time it was consumed. This was happening so frequently and the money loss was so annoying that it was decided to call on the bacteriologist for assistance with the problem. Before taking up the study it was thought best to look up what previous published work was available along these lines and it was surprising how little could be found on so important a matter. This was probably due to the fact that dairy publications and Agricultural Experiment Station bulletins are not very widely circulated outside of those particularly interested.

The presence of molds or yeasts was what this study was undertaken for primarily, so literature on this subject was especially consulted. It was found that this subject had been discussed at the 16th Annual Meeting of the Wisconsin Butter Makers' Association[1] and that W. B. Combs and C. H. Eckles[2] had also done considerable work upon it.

In none of the literature available was a technic given for such work as we were to undertake, so a method had to be developed. While engaged in this, a study was made of the operation of the plant; here it was found that the raw cream from many farms and of varying ages was all poured into one large vat, steam being used to remove the last of the cream from the cans. From this vat the cream was pumped into the forewarmer where it is heated to between 90° and 100° F. and its acidity tested. While in this stage of the process sufficient milk of magnesia suspension is added to bring the total acidity down to between 0.35 per cent and 0.4 per cent. After thorough mixing the cream runs by gravity to the pasteurizer, where its temperature is raised to 148° F. The cream takes one minute to pass through this step in the process, finally reaching the holding vat, which it enters through a strainer, and it remains here twenty minutes, a series of baffles insuring thorough mixing and uniform exposure to the heat.

From here at a temperature of 142° F. the cream is elevated to the top of the cooling coils, through which cold water is pumped, and after flowing over these enters either the ripener vats or the churn at a temperature of 65° F. The cooling coils are not enclosed, neither is the holding vat covered. Mention has been made of the "ripener" vats. These are large tin-lined boxes with a capacity of 1,000 gallons of cream in which, if "starter" is used, the lactic acid culture is added and the whole mixed well and allowed to stand at a temperature of 52° F. for two hours in order that the lactic acid

*From the North Dakota Public Health Laboratories, Grand Forks, North Dakota.

bacteria may multiply and raise the acidity in order that the bacteria which may have missed death in pasteurizing will be destroyed and the cream will also churn better.

"Starter" is the name given to the lactic acid culture which is prepared fresh each week from a pure culture received from a dairy supply house. The method of preparation is the familiar one used in homes where pancake batter is always kept in a jar behind the stove. A little of the active principle is added to a batch of pasteurized sweet milk, in this case a pure culture of lactic acid bacilli, allowed to grow overnight at room temperature and the resulting mixture is run into ten gallon cans and used as a "starter" for the big tanks described above.

To return to our cream. As has been said after leaving the cooler it may go to the ripener or to the churn. The cream is churned in large rotary churns for one hour, salt and coloring matter being added during the process, salt and moisture determinations being made before the product is finished. The butter is removed from the churns by hand and packed in tubs or pound cartons being then ready for sale.

A study of the equipment showed that there was no cover to the holding vat and that the cooler was open on all sides, flies and dust having free access. In busy times when the plant was run to capacity, 30,000 pounds of butter were produced, two shifts being employed. It was found also that the employees were quite likely to neglect to thoroughly clean the complete system and that leaks in the pipes allowed dirty water in and cream to run out. The ceiling of the main work room was of typical factory construction being actually the floor and supporting joists of the floor above which had been whitewashed at one time, but was now spotted with what proved on examination to be, a growth of black mold.

After some consideration of the matter, it was decided to make bacterial counts on the cream at the various stages in the butter making process and at the same time make cultures for the presence of molds. Plain agar made according to the directions contained in the A. P. H. A. Standard Methods for the Examination of Milk was used for the plate count and a 1 per cent maltose agar was used for the fungi. The agar plates were counted after 48 hours in the incubator at 38° C. and the maltose plates were examined after four days in a dark drawer at laboratory temperature.

Samples were taken from the cream, entering the pasteurizer, entering the holding vat, leaving the holding vat, bottom of the cooling coils, ripener, entering the churn, buttermilk, butter. Before proceeding with the results of these tests in detail, it is interesting to note that the commercial value of butter is determined largely by its flavor and aroma. These two items are due to the by-products formed by the fermentation of the milk and cream and held in combination with the fat. If, therefore, the bacteria and other organisms present produce a disagreeable result, the keeping qualities of the butter and its flavor and aroma will be affected also. It is thus manifestly the duty of the buttermaker to so control his product that only the desirable bacteria shall predominate.

It was found in preliminary tests that the cream was handled so carelessly by the ordinary farmer that it was necessary to make dilutions of 1-10,000 in making the plate counts. The following table is typical of many of the results obtained for the first few months.

TABLE I

NO.	SAMPLE COLLECTED FROM	DILUTED 10,000 TIMES 1 C.C.		TEMPERATURE	ACIDITY
		AGAR	MALTOSE	F	%
5	Entering pasteurizer	230,000 1 mold	2 white molds; 3 green center molds	157	0.40
6	Entering holding vat	60,000 1 mold	1 mold; green center	156	
7	Leaving holding vat	10,000	1 white mold; 2 green center molds	154	
8	Bottom cooling coils	40,000	1 white mold; 1 green center mold	81-82	
9	Entering ripener	20,000	5 green center molds; 4 white molds	64	
13	Entering churn	2,260,000	2 white molds; 2 green center molds		
14	Buttermilk	4,790,000	2 blue center molds; 5 white molds; 6 green center molds		
	Butter				

At the time this investigation was begun "starter" was being used. Examination of the "starter" as it was prepared for use in the ripener vats showed molds to be present, so that if through careful operation of the pasteurizer and maintenance of an even temperature in the holder and careful attention to the pipes and cooling coils, the cream was free from these when it arrived at the ripening vats, all the good work was undone and the molds would still be present in the butter. In every case where the raw cream was cultured the bacterial count was very high and molds were present, so thorough cleaning of the whole system and care in operation was imperative.

At many different times the plates were shown to the employees and a lively interest was displayed. During the slack time there was a marked improvement in the cleanliness of the final product.

It was found that by giving the whole system a thorough steaming out before beginning the day's run that the counts were kept down and the cream didn't lose any of its heat while passing through its various stages; in order that the operator might know how the temperature of the holding vat was being maintained a Tycos recording thermometer was installed. Instruction was then given in the preparation of the "mother" starter. It was found that some of the sour cream from a previous day's run was used to make up another batch of starter and after several lots were made up in this way, a large number of objectionable bacteria would be present along with the original lactic acid culture. No definite temperature was maintained, the jars being kept between 60° and 70° in summer and between 70° and 72° in win-

ter and when the contents of the jar had coagulated the material was ready for use. This usually took seven to eight hours.

As no incubator facilities were present, it was rather difficult to devise a means to carry on the bacterial growth under methods used in a laboratory but by care in placing the jars near a steam pipe a higher temperature was obtained. Greater care was also advised in the way the milk was handled for the starter, attention being directed particularly to the pasteurization, as the milk is delivered in a filthy condition.

However, the method of using "starter" in the butter making process was discontinued and the problem of handling that item was thus disposed of.

Table II shows the condition of the cream after three months' intensive study and effort to get the working conditions improved, the ceilings, pipes, and overhead machinery cleaned, and the men trained in the habit of keeping the pipes and machinery steamed out daily and the covers closed on the ripener vats.

TABLE II

NO.	SAMPLE COLLECTED	DILUTED 10,000 TIMES 1 C.C. AGAR	MALTOSE	TEMPERATURE F	ACIDITY %
5	Entering pasteurizer	1,160,000 many fine	spreaders	150-155	.43
6	Leaving pasteurizer	18,900	1 green center mold	154	
7	Leaving holding vat	8,100	1 spreader	147	
8	Bottom cooling coils			62-64	
9	Entering ripener vat	60,000		64	
13	Entering churn	10,000			
14	Buttermilk	40,000	1 spreader		
	Butter				

Glancing at this table a marked improvement is noticed in comparison with Table I the bacterial count and mold content being greatly lowered.

During the winter months the amount of work was greatly lessened and the cream arrived in good condition as regards temperature, many cans being frozen almost solid. Greater care was also taken in the handling as only cream enough for one or two churns a day was being received. Table III is characteristic of the results at this time.

TABLE III

NO.	SAMPLE COLLECTED	DILUTED 10,000 TIMES 1 C.C. AGAR	MALTOSE	TEMPERATURE F	ACIDITY %
5	Entering pasteurizer	970,000 innumerable fine	3 yeasts 1 mold	152	.43
6	Leaving pasteurizer	10,000	1 green center mold	151	
7	Leaving holding vat			149	
8	Bottom cooling coils			58-61	
9	Entering ripener	30,000		61	
13	Entering churn	20,000			
14	Buttermilk	80,000			
	Butter				

These results follow those of Table II very closely and show what can be done when care is exercised.

After the work had been carried on for over a year another technic for sampling was adopted, namely, that of taking many small quantities from different points during the whole of the run and so making a representative composite sample of the cream at each stage in its treatment. Prior to this time but one sample was taken from each place during the course of the run, and in that way it could not be described as representative. Table IV describes the manner in which the work was handled under the changed method.

TABLE IV

	BACTERIA PER 1 C.C. DILUTIONS			AVE. PER	MOLDS PER 1 C.C. DILUTIONS		
SAMPLE	1:1000	1:100	1:20	1 c.c.	1:1000	1:20	Ave.
Raw cream	1,200,000			1,175,000	48,000		48,000
1st Comp. hold. vat	150	1000	960	980		60	60
Last " " "		1500	1400	1450		120	120
Cooler		2000	1600	1800		160	160
Churn Sam. No. I		3000	2400	2700		260	260
Churn Sam. No. II		2000	1700	1850		120	120
Buttermilk	30,000	32000		31000		280	280
Butter		8200	6500	7350		40	40

Samples 1, 2, 3, and 4 are representative of samples taken at intervals of 15 minutes during entire run from 8:30 A.M. until 11:30 A.M. Churn sample No. 1 was made up of six samples, three from each of two churns; churn sample No. 2 was made up of nine samples, three from each of three churns. The buttermilk was taken from the first churn of churn sample No. 1 as was also the sample of butter. These results are slightly high in that they show gradual increase after leaving the pasteurizer. The averages also show the enormous increase in bacteria in the buttermilk. This point should be kept in mind and followed through the data for each week.

Altogether six such comprehensive examinations were made over a period of six weeks, 43 samples in all being included in this study. By this method it was possible to arrive at an idea as to the amount of bacterial and mold removal. Table V shows this nicely.

TABLE V

	BACTERIA PER 1 C.C.		MOLDS PER 1 C.C.	
SAMPLES	1	2	1	2
Raw cream	767111	1094166	70800	45416
1st Comp't H. Vat	3700	1470	2010	39
Last Comp't H. Vat	2380	2362	1100	29
Cooler	1220	3298	1040	37
Ave. of Churn samples	2740	3565	830	51
Buttermilk	41100	36166	4950	113
Butter	6100	4958	855	24
Percentage of removal	99.01%	99.55%	98.76%	99.96%

No. 1=Samples collected from Nov. 17 to Dec. 31, 1920.
No. 2=Samples collected from Jan. 21 to Feb. 25, 1921.

There are several things of interest which may be pointed out in the discussion of the above data.

First.—A direct comparison of the average number of bacteria per one

cubic centimeter (Table V) in the samples collected from Nov. 17 to Dec. 31, 1920, with those of the later collections, reveals the interesting fact that although the raw cream gave a count of approximately 31,500 per 1 c.c. less than that obtained for the last collection, it also gave an end product having a count of over 1000 bacteria per 1 c.c. higher than that obtained for the later samples. In the same manner, a direct comparison of the number of molds per 1 c.c. of No. 1 with No. 2, at first sight seems to show that no great reduction has been accomplished. However, a closer study shows the reduction to have been much greater. On a directly proportional basis, if in No. 1 the raw cream with a count per 1 c.c. of 70,800 gave an end product with a count of 855, then No. 2 should give an end product having a count of 548. A glance at the table shows the count to be only 24. The percentage of removal, which is an index of plant operation, shows the same reduction. The question now naturally arises as to whether this is a true reduction or merely a "happen-to-case." As to the bacterial count, there is no doubt but that there has been a substantial reduction. As to the molds, the reduction is not so well grounded, because in some instances the dilutions were too high. Personally we do not think much weight should be attached to this objection, for even after making undue allowances, the reduction still remains very noticeable. It is evident therefore, that since there has been a substantial reduction, it can be explained only in two ways, namely, increased efficiency in plant operation, or smaller amount of raw product handled. If the amount of raw cream were checked for these two periods we believe one would find that the amount of raw product per day would be less for Jan. 21 to Feb. 25, 1921, than for the period represented by the other samples. But, after making due allowance for this fact, it must still be borne in mind that for the last period, the plant was in operation every other day, a factor which would more than offset the above argument. The only logical conclusion, therefore, is better plant operation, and from observations, we are convinced that this is the correct conclusion.

Second.—The manner of collecting the various samples is very satisfactory. The results given by the method of collection are very reliable and representative of actual conditions. The manner in which the raw cream is handled before it enters the pasteurizer also insures a thorough mixing before samples are taken. These samples which are taken at intervals of 15 minutes and from four different stations, give a good history of what happens to the raw cream before it is placed in the churns. Composite samples from the churns, buttermilk and butter make these samples representative of these stages.

Third.—The percentage of removal has been increased over that obtained for the period from Nov. 17 to Dec. 31. In case of removal of bacteria this increase is .54 per cent; in case of removal of molds, it is 1.2 per cent. Although these are apparently small, in reality they are large because after the removal has reached 99.00 per cent, 0.1 per cent means quite a noticeable increase.

Fourth.—The results for this period show what has been thought for

some time to be a fact, namely, a gradual increase in count after leaving the pasteurizer.

Fifth.—The results for each inspection, as well as the final average, conform to previous findings in that there is a tremendous increase in number, both in molds and bacteria, in the buttermilk. Of the samples collected and reported, there has been found no exception to the above statement. If this increase confined itself to the buttermilk alone, it would not be necessary to consider it, but as a matter of fact the increase is also noticeable in the butter. Reference to Table V which is representative of fifteen different inspections, shows that the count of bacteria is higher in butter than it is in the cream as it enters the churn; with the number of molds the difference is not so marked. Even so, should we add the count of the buttermilk with that of the butter, both of which are the end products of the cream, we find that the number of bacteria has been increased 13 times that found in the cream as it enters the churn, while the average increase in the molds is about five times the original number.

Nothing has been said concerning the finished product, the butter. When these studies were begun samples of butter were taken in sterile bottles and stored in the ice box for from six to eight weeks which was considered, according to office records, to be the interval which elapsed between the time the butter left the creamery until it was consumed. The object was to see whether objectionable odors, or flavors would develop and whether molds would appear. These samples were collected; possibly weekly, for one year and, with but one or two exceptions, stood the test very well. However with the improved technic adopted latterly, plates were made of the butter also.

At all times it has been noted that the buttermilk has had a high bacterial and mold content. The theory put forward by one of us and one which we feel sure will be accepted by all, is that in the process of churning all the clumps of bacteria and mold spores are broken up and so are given a greater chance to develop and so raise the count.

CONCLUSION

After two years' study, while the work is still incomplete and this can be called but a preliminary report, it is quite evident that by careful, efficient operation of the pasteurizer and cooling systems it is possible to produce commercially a good quality of butter of low bacterial content and quite free from molds at the time it is churned.

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THE SYNTHESIS OF ARSPHENAMINE AND A STUDY OF SOME OF ITS INTERMEDIATE DERIVATIVES

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(*Continued from page* 165)

TOXICITY STUDIES

IT HAS been observed that toxic symptoms frequently make their appearance after the administration of arsphenamine. Whether this is due to an idiosyncrasy on the part of the patient, lack of the observance of proper precautions in the preparation and injection of the solution, to impurities present in the manufactured product, or to other conditions, has not been sufficiently determined to date.

The following toxicity studies were undertaken to determine to what extent, if any, undue toxicity may be attributed to the presence of certain known compounds or variations in the commercial product, and to possible contamination with certain intermediates and by-products formed in the process of manufacture.

Arsenoxide (3-amino-4-hydroxyphenyl-arsenious oxide).—All commercial samples of arsphenamine contain a small amount (0.4 to 5.0 per cent) of material which is not precipitated from methyl alcohol by calcium carbonate. This material has been commonly referred to in the literature as "arsenoxide." A statement also occurs in the literature to the effect that this compound is twenty times as toxic as arsphenamine. The latter appears to have originated through reference to an article published by Ehrlich and Bertheim in the *Berichte der deutschen chemischen Gesellschaft.* In the monograph by Ehrlich and Hata on the chemotherapy of the spirilloses, in which the data upon which this statement is apparently based are presented in detail, the information conveyed is that the tolerated dose for chickens infected with spirilloses is twenty times as great as the curative dose. For the information of the reader both articles are quoted here.

"The hydrochloride of diamino dihydroxy-arseno-benzene shares with other arsenic compounds the peculiarity that it is easily oxidizable. When it is exposed to the air, it will quickly form aminohydroxyphenylarsenoxide; however, the formation of an arsenoxide compound takes place immediately, if the experiment is carried on in the usual salt bottle. This fact is therefore of the greatest significance in the practical application of the substance as a curative agent, because the aminohydroxyphenylarsenoxide is about 20 times as toxic as the pure hydrochloride of an arseno compound. It is possible through the use of the different degrees of solubility of arseno and of the arsenoxide base to determine approximately the arsenoxide content of the preparation. (Ehrlich and Bertheim, Berl. deutsch. Chem. Gesellsch., 1912, xlv, p. 764.)

"Dosis tolerata pro kilogram of chicken: 0.03 g. Amidophenol arsenoxide shows also the very good effect of this substance upon the diseased animal even much more so than upon the healthy animal. (Table xxxii.)

"If we now look over the above mentioned curative experiments with chickens infected with spirillosis, the relation between the curative dose and the tolerated dose of the single substances results as follows:

Atoxyl	0.03 / 0.06	C / T	1 / 2
Arsacetin	0.03 / 0.10	C / T	1 / 3.3
Arsenophenyl glycin	0.12 / 0.40	C / T	1 / 3.3
Arsanylchloride Quicksilver	0.04 / 0.100	–	1 / 2.5
Dioxydiamidoarsenobenzene	0.0035 / 0.200	C / T	1 / 58
Amidophenolarsen oxide	0.0015 / 0.030	C / T	1 / 20

"From the same comparison it appears that the first four remedies acted more unfavorably than the last two; of these again the dioxydiamido arsenobenzol is better than the amidophenolarsen-oxide, a fact that I was able to confirm with my experiments of relapsing fever." (Ehrlich and Hata, Die experimentelle Chemotherapie der Spirillosen, 1910, p. 57.)

In order to clear up the question of the toxicity of this compound, a quantity of the material was prepared and tested. Arsenic and nitrogen determinations showed that the material consisted of approximately 91 per cent of arsenoxide hydrochloride and corresponded to the formula

$$HCl,\ NH_2C_6H_3(OH)AsO,\ \tfrac{1}{2}C_2H_5OH.$$

The product which was produced for use in this experiment contained alcohol of crystallization and the calculation for the hydrochloride is obtained as follows:

$$\frac{HClH_2NC_6H_3OHAsO}{HClH_2NC_6H_3OHAsO\tfrac{1}{2}C_2H_3OH}$$

Table I shows the results of the toxicity tests made by Dr. G. B. Roth. The tests were made on rats, the material being injected in alkaline solution.

TABLE I

TOXICITY OF ARSENOXIDE

DOSE IN MG. PER KILO	NUMBER OF ANIMALS USED	LIVED	DIED
10	5	5	0
15	5	3	2
20	5	2	3
30	5	1	4
40	5	0	5

These results show that the tolerated dose for rats lies between 20 and 30 mg. per kilo. Similar tests conducted at this laboratory with arsphenamine have demonstrated the tolerated dose to be in the neighborhood of 100 to 120 mg. per kilo. These figures correspond very closely with those obtained by Ehrlich and Hata for chickens, rats and rabbits quoted above. It may, therefore, be stated that arsenoxide is only about 6 to 7 times as toxic as arsphenamine. Furthermore, it can be stated with a fair degree of certainty that the abnormal toxicity frequently observed in the case of arsphen-

TABLE II

RELATION OF ARSENOXIDE CONTENT OF ARSPHENAMINE TO TOXICITY VALUES

PRODUCT	CONTROL NUMBER	PER CENT OF AS	PER CENT OF ARSENOXIDE
A			
	1	31.59	1.0
	2	31.22	0.9
	3	31.10	0.8
	4	31.27	0.6
	5	31.31	0.9
	6	31.50	0.5
	7	31.22	0.7
	8	31.31	1.7
	9	. . .	0.7
	10	31.12	1.0
	11	31.31	0.6
	12	31.50	0.5
	13	31.25	0.5
	14	30.11	0.7
	15	31.23	0.6
	16	30.75	0.5
	17	30.70	0.8
	18	30.75	0.8
	19	30.85	0.7
	20	31.12	0.8
	21	31.46	0.7
	22	. . .	1.1
	23	31.29	1.7
	24	30.60	0.5
	25	31.15	0.9
	26	30.78	0.6
	27	30.19	0.8
	28	31.25	1.0
	29	30.94	0.7
	30	31.12	1.0
	31	31.05	0.7
	32	31.09	0.7
	33	30.75	0.6
	34	31.05	1.2
	35	31.50	0.4
	36	31.44	0.5
	37	31.12	0.7
	38	31.15	0.8
	39	30.90	0.9
	40	30.63	1.8
	41	30.91	2.3
	42	31.58	1.9
	43	32.15	2.9
	44	31.97	1.9
	45	31.65	1.9
	46	30.70	1.4
	47	. . .	1.3
	48	30.92	1.5
	49	31.55	1.5
	50	30.93	1.3
	51	31.06	1.9
	52	31.48	. .
	53	30.15	. .
	54	30.30	0.8
	55	31.00	0.7
	56	31.12	0.7
	57	30.93	0.8
	58	31.20	1.6
	59	30.92	2.3
	60	31.29	2.6

TABLE II—(CONTINUED)

PRODUCT	CONTROL NUMBER	PER CENT OF AS	PER CENT OF ARSENOXIDE
Product B			
	1	30.54	0.7
	2	30.73	0.8
	3	30.95	0.6
	4	30.57	1.0
	5	30.49	0.8
	6	30.26	0.7
	7	. . .	0.7
	8	29.89	1.3
	9	30.88	1.1
	10	31.01	1.8
	11	30.88	1.6
	12	30.88–	1.6
	13	30.46	1.6
	14	30.65	1.6
	15	30.65	1.8
	16	30.65	1.1
	17	31.31	1.5
	18	31.01	1.8
	19	29.71	1.1
	20	30.75	1.0
	21	31.01	0.9
	22	29.89	1.4
Product C			
	1	30.95	1.8
	2	29.88	1.7
	3		1.9
	4	30.07	4.1
	5	30.30	1.7
	6		1.6
	7	30.53	1.3
	8	30.40	1.8
	9		1.8
	10	30.23	1.6
	11	30.49	2.3
	12	30.40	1.3
	13	30.06	2.0
	14		0.9
	15	29.75	1.5
	16		1.4
	17		1.4
	18		2.0
	19		1.1
	20		1.6
	21		2.3
	22	30.57	1.1
	23		1.4
	24	29.71	1.1
	25		1.4
	26	29.51	1.1
	27	30.75	0.8
	28	30.95	0.7
	29	30.88	1.1
	30	30.18	0.7
	31	30.75	0.7
	32	31.10	1.1
	33	30.88	1.2
	34		0.9
	35		0.7
	36		1.1
	37	31.47	1.1
	38	30.77	0.7
	39	30.94	0.9

amine is not due in any marked degree to the presence of this compound, inasmuch as numerous tests carried out in this laboratory indicate that there is no apparent relationship between the "so-called" arsenoxide content and degree of toxicity. Preparations with a relatively high arsenoxide content were found to give low toxicity values and *vice versa*. Table II contains data of this nature.

Methyl Alcohol.—Attention has been directed to the presence of methyl alcohol as a possible cause for abnormal toxicity. It should not be understood that the methylated salvarsans are not being referred to in this connection, but a product in which the methyl alcohol is mechanically held or is present as "so-called" alcohol of crystallization. A preparation of this nature (HCl, $NH_2C_6H_3(OH)As.)_2.CH_3OH$) obtained by reduction with sodium amalgam is described by Ehrlich and Bertheim (1912).

During the course of the examination of commercial samples of arsphenamine in this laboratory, a number of tests were made with Schiff's reagent to detect the presence of free methyl alcohol or alcohol loosely combined. The results obtained, however, were negative in all cases. Later, 400 g. of the arsphenamine were subjected to distillation and then refractionated with the result that less than 1 c.c. of methyl alcohol was obtained in the distillate. Even though this amount does not represent the total quantity present, a portion probably being lost through oxidation, it is highly improbable that there is a sufficient amount of methyl alcohol present to account for any undue

TABLE III

HYDROGEN-ION CONCENTRATIONS OF SOLUTIONS OF THE ACID AND DISODIUM SALTS

MANUFACTURER	CONTROL NO.	P+H ACID SALT	P+H DI-NA SALT	C.C. N/1 Na_2OH ADDED TO FORM THE DI-SALT
Product A				
	1000	4.8	9.4	1.44
	1239	5.1	9.4	1.47
	1208	5.0	9.4	1.70
	1303	5.2	9.4	1.72
	1294	5.0	9.4	1.36
	1288	5.2	9.4	1.52
	1292	4.6	9.5	1.36
	1300	4.4	9.3	1.50
	1298	5.0	9.2	1.45
Product B				
	H 28	5.0	9.4	1.72
	H 30	5.0	9.2	1.76
	H 31	4.9	9.3	1.80
	H 34	4.9	9.4	1.74
	H 37	5.0	9.3	1.72
	H 40	5.0	9.4	1.80
	H 51	4.9	9.2	1.70
Product C				
	A 22	4.6	9.4	1.50
	A 24	4.8	9.4	1.47
	A 25	4.8	9.6	1.47
	A 34	4.5	9.6	1.50
	A 37	4.7	9.4	1.62
Product D				
	3	4.8	9.2	1.47
	5	5.0	9.4	1.57

toxic action. In fact, when salvarsan first began to be used, it was recommended in the directions for preparing solutions for injection, that a small amount of methyl alcohol be poured over the material before the addition of the water in order to facilitate solution.

Variations in Degree of Acidity.—Hydrogen-ion determination made in connection with the testing of commercial samples of arsphenamine show that the hydrochloric acid content of the different preparations on the market varies to a considerable extent. This variation is thought to be the cause for the alkalinity troubles which are reported from time to time, and may be a factor indirectly influencing toxicity. It is evident that, if the hydrochloric acid content varies and the same amount of alkali is added in all cases, the hydrogen-ion concentration of the finished solutions will vary. As this condition may be an important factor influencing the formation of precipitates in the blood, its importance in this connection is readily understood. Data of this nature for solutions of the acid salt and disodium salt, using methyl red in the acid range and cresol phthalein and thymol blue in the alkaline

TABLE IV

TOXICITY OF INTERMEDIATES AND BY-PRODUCTS

COMPOUND	PROPERTIES GENERAL	RAT	DOSE IN MGS.	DIED IN	LIVING AT END OF	REMARKS
3-amino-4-hydroxy-phenyl-arsinic acid	Light yellow powder, insol. in H_2O, sol. in dil. NaOH. Sol. with a light brown color.	1904	400	46 hrs.		
		1903	300		19 days	
		1905	200		" "	
		1906	100		14 "	
oxalyl-4-amino-phenyl-arsinic acid	Purplish-gray powder, insol. in H_2O, readily sol. in weak NaOH, forming a purplish colored sol.	1962	400		13 days	All in good condition
		1963	300		" "	
		1964	350		" "	
		1965	250		" "	
		1966	200		" "	
3-nitro-4-amino-phenyl-arsinic acid	Deep yellow powder insol. in H_2O, sol. in dil. alkali, darkens as alkali is added forming deep mahogany-col. sol.	116	150	20 hrs.		
		115	140	3 days		
		113	140	2 days		
		114	130	4 days		
		112	110		9 days	
		92	100	4 days		
		94	90		9 days	
		93	80		9 days	
		95	70		9 days	
		96	60	8 days		
3:5-dinitro-4-hydroxy-phenyl-arsinic acid	Fine light yellow powder, partially sol. in H_2O, forming a yellow colored sol. On making alkaline, the color changes to orange.	2065	50		14 days	
		1982	250		11 "	
		1983	300	5 hrs.		
		1984	200	9 hrs.		
		1985	150		11 "	
		1986	100		11 "	
		1972	400	6 hrs.		
3:5-dinitro-4-amino-phenyl-arsinic acid	Dark brownish yellow powder, insol. in H_2O, Sol. in dil. NaOH, forming a dark brown sol.	1973	350	21 hrs.		
		1974	300	3 days		
		1975	250	22 hrs.		
		1976	200	3 days		

range, are given in Table III. The values given represent the hydrogen-ion concentrations of 2 per cent solutions of the acid and disodium salts, respectively, this being the concentration usually used in injecting rats for toxicity tests. These tests are comparable but more recent work shows that these are not absolute values.

Intermediates and By-products.—Several investigators have called attention to the possibility of contamination of arsphenamine with certain intermediates and their derivatives, and have assumed that such contamination may be the cause of the abnormal toxicity sometimes apparently manifested by the commercial product. In order to test the correctness of this assumption, a considerable number of intermediates and by-products were isolated or prepared, and such toxicity tests carried out as conditions permitted at the time. The results of these tests show that it is improbable that the relatively small quantities of these substances, which may possibly be contained in the finished product, are the cause of abnormal toxicity.

The solutions of intermediates for injection were prepared in the same manner as in the case of arsphenamine, i.e., 1.5 N/1 NaOH were added for every 200 mg. of the compound dissolved. Later investigations showed that 0.84 c.c. N/1 or 4 per cent NaOH was the proper amount of alkali to use for intravenous administration.

SUMMARY

The descriptions of the processes given above show that there are three well defined methods by which "nitro oxy," the mother substance of arsphenamine, may be prepared. Attention has also been directed to the fact that the former, "nitro oxy," exhibits a peculiar type of dimorphism, which necessitates the exercise of extreme care in its preparation if the type best suited for reduction is to be obtained. Another important point which has been brought to notice is the importance of eliminating by-products from the intermediate substances, notwithstanding the statements to the contrary made by some inexperienced investigators, who contend that these products will be removed at some later stage in the process.

The foregoing descriptions of processes of manufacture have shown further that there are two well-defined procedures for reducing "nitro oxy" to arsphenamine base, namely, the progressive and direct methods. It may also be stated that it is the latter method, or some modification thereof, which is usually followed in the preparation of arsphenamine on a commercial scale. The modification most commonly made consists in the addition of magnesium chloride to the "hydrosulphite" mixture for the purpose of guarding against overreduction and sulphur compounds.

A method for converting the free base into the dihydrochloride of arsphenamine has also been described. It should be stated here, however, that the process is not so simple as it appears at first glance, and that the exact technic followed in commercial practice is more or less known only to the manufacturers. It may be said in addition that the care exercised at this point is an important factor influencing the purity of the final product. "Hydrosul-

phite'' contains metallic zinc and other inorganic impurities, which may be filtered off simultaneously with the free base and subsequently dissolved in the methyl alcohol-hydrochloric acid mixture if the proper precautions are not observed.

With respect to the toxicity of the preparations on the market at the present time, it may be said that the average American product is somewhat less toxic than that which was formerly produced by German manufacturers. Occasionally, however, even the American product exhibits an abnormal toxicity as shown by the routine tests on animals. This is probably due to the presence of physical conditions which have not been definitely determined to date. The results of the toxicity studies described above, however, are evidence to the effect that it is not due in any appreciable degree to the presence of arsenoxide, methyl alcohol, or the following intermediates and by-products: 3-amino-4-hydroxyphenylarsinic acid, oxalyl-4-aminophenylarsinic acid, 3-nitro-4-aminophenylarsinic acid, 3:5-dinitro-4-hydroxy-phenylarsinic acid, 3:5-dinitro-4- amino-phenylarsinic acid. Arsenoxide, although 6 to 7 times as toxic as arsphenamine, has been found in such small quantities (0.4 to 3.0 per cent) that it can hardly be considered an important factor in this connection. Furthermore, the toxicity tests carried out indicate that there is no noticeable relationship between the degree of toxicity of arsphenamine and its ''arsenoxide'' content. Determinations made of the methyl alcohol content show that it is also present in such small quantities that it is a negligible factor. With one possible exception, 3-nitro-4-aminophenylarsinic acid, the intermediates mentioned are less toxic than arsphenamine.

The variations observed in the hydrogen-ion concentrations of solutions of commercial samples of arsphenamine prepared by different manufacturers, using a fixed amount of alkali, indicate that the hydrochloric acid content may be a factor influencing toxicity. Animal experimentation in this field, however, will be necessary before accurate conclusions can be drawn.

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AN EXPERIMENTAL INVESTIGATION OF THE PHARMACOLOGIC ACTIVITY OF DRUG STORE SAMPLES OF INFUSION OF DIGITALIS, U. S. P. IX*

By A. Richard Bliss, Jr., Phm.D., M.D., Atlanta, Ga.

BECAUSE of the frequent complaints of clinicians concerning the unreliability of infusion of digitalis, U. S. P., and the meager available data concerning the relative activity of this preparation, particularly the preparation as it is found in the average retail pharmacy, the writer undertook the investigations the results of which are herewith presented.

The United States Pharmacopeia gives biologic assays for digitalis,[1] fluidextract of digitalis,[2] and tincture of digitalis,[3] but gives no method for standardizing infusion of digitalis.[4] One reason for the omission of a biologic assay in the case of the infusion is indicated in the pharmacopeial instructions *"Infusion of digitalis must be freshly prepared from the leaves,"*[5] it being doubtless taken for granted that an infusion *freshly* made according to the pharmacopeial method from standardized leaves will represent the activity of the amount of standard leaves employed. The results of this study will be of interest to those who formulated the foregoing conclusion.

Some years ago many schools taught that the infusion of digitalis was a more active diuretic than the tincture and the fluidextract, explaining this supposed difference on the ground that the several active constituents of the crude drug are extracted in different relative proportions by the menstrua employed in the infusion[6] (water), the fluidextract[7] (5 of alcohol to 1 of water—by volume), and the tincture[8] (3 of alcohol to 1 of water—by volume). Although clinical experiences, animal experimentation, and the physical properties of the active constituents of digitalis long ago showed the errors of such belief, there are many practitioners who are seemingly firm believers in this "theory." Digitoxin, digitalin, and digitalein, the three glucosides that supposedly represent the activity of digitalis, are all soluble in alcohol. Digitoxin, which according to Bastedo[9] and others most nearly represents the digitalis actions, is practically insoluble in water; digitalin is slightly soluble in water; and digitalein is soluble in water. Cushny[10] states that infusion of digitalis contains only traces of digitoxin. Sollmann[11] states that a 1:10 infusion (not U. S. P.) contains ⅔ of the digitoxin of the leaves. A fourth principle, a saponin body called digitonin, is soluble in water, and is said by Bastedo[12] and others to possess the property of holding the otherwise water-insoluble (or slightly soluble) glucosides of digitalis in aqueous solution. It is also stated that digitonin, by the intravenous method, is a physiologic antagonist to digitoxin, but that it is unabsorbable by the alimentary tract. Kiliani[13] claims

*From the Laboratories of Pharmacology of the School of Medicine of Emory University.
Received for publication, June 25, 1921.

that the crude drug contains but traces of digitonin. Bastedo[14] says he has frequently seen the infusion given in one-half ounce doses, the equivalent of 36 minims of the tincture. The writer has seen one ounce doses of the infusion (U.S.P. VIII) administered without subsequent toxic symptoms.

Hatcher and Eggleston[15] showed that an infusion made by pouring 1000 c.c. of boiling water onto 10 grams of digitalis (No. 60 powder), allowing it to stand in a boiling water-bath for one hour with frequent stirring, and filtering while hot, retains its activity with little loss for several weeks. Pittinger and Mulford[16] showed an average loss of 47.8 per cent in five samples, 8 months old, of a 50 per cent alcohol, not defatted tincture; 22.8 per cent loss in five samples, 8 months old, of a 50 per cent alcohol, fat-free tincture; and 40.7 per cent loss in five samples, 8 months old, of an 80 per cent alcohol, fat-free tincture. They present no data concerning the infusion. Hale[17] found official fluidextracts to have lost only an average of 6.6 per cent in two years. Roth[18] found an average loss of activity in seven samples of fat-free tincture of 14 per cent in six months; two of his samples showing no loss, while two others showed a very high loss. Houghton and Hamilton[19] showed an average yearly loss of 8 per cent in eleven samples of extract five years old; 4 per cent average yearly loss in eight samples of fluidextract, (U.S.P. VII,) six years old; 10 per cent average yearly loss in eleven samples of fluidextract, (U.S.P. VIII,) three and one-half years old; and 9 per cent average yearly loss in eight samples of tincture three years old. They concluded that a maximum average loss of 10 per cent per year can be expected in the tincture and the fluidextract. Goodall[20] states that probably tincture of digitalis retains its full activity for one year. Hamilton and Rowe,[21] following a series of experiments with the tincture which showed a loss of from 0 per cent (with fat-free preparations) to 43 per cent, the ages running from 5 months to 8 months, concluded that the degree of deterioration varies with different lots, that a fat-free tincture made with 70 per cent alcohol is less subject to deterioration, and that the deterioration of the tincture is not as uniformly rapid as isolated experiments would indicate. Bastedo[22] states that under the influence of heat or acids, or when kept for some time in aqueous solution, as in the case of the infusion, the glucosides of digitalis tend to decompose and may form toxiresins which have a central convulsant action. None of the foregoing investigators, with the exception of Hatcher and Eggleston, present data concerning the stability and the relative activity of the infusion.

THE METHOD

The method of biologic standardization used in this investigation is that known as the Hatcher and Brody Cat Method.[23]

THE SAMPLES

The samples investigated were obtained from various retail pharmacies.

THE RESULTS

The results of the investigation are presented in tabulated form. The values in the last column of the table represent the activities of the samples

expressed as percentage of the theoretical activity as calculated from the amounts of standardized drug supposedly used.

TABLE I

TABLE SHOWING THE RELATIVE ACTIVITY OF VARIOUS SAMPLES OF INFUSION OF DIGITALIS OBTAINED FROM RETAIL PHARMACIES

The values in the "Average Activity" column express the percentages of the theoretical activity as calculated from the amount of crude drug employed or supposed to have been employed. Fractions are omitted.

SAMPLE	METHOD OF PREPARATION	NUMBER OF ESTIMATIONS	AVERAGE ACTIVITY (PERCENTAGE OF THEORETICAL ACTIVITY)
1	U. S. P. IX	4	31%
2	U. S. P. IX	4	42%
3	Dilution of Fluidextract	4	60%
4	Dilution of Fluidextract	3	65%
5	U. S. P. IX	4	33%
6	U. S. P. IX	4	51%
7	U. S. P. IX	3	29%
8	Dilution of Fluidextract	4	63%
9	Dilution of Fluidextract	4	60%
10	U. S. P. IX	4	37%
11	U. S. P. IX	4	40%
12	Dilution of Fluidextract	3	65%
13	U. S. P. IX	3	42%
14	U. S. P. IX	4	41%
15	U. S. P. IX	4	35%

SUMMARY

Fifteen samples of infusion of digitalis, selected at random from retail pharmacies, showed an average activity of but 46.26 per cent of the theoretical activity calculated from the amount of standardized drug supposedly used in the manufacture of the infusion.

Five of the fifteen samples, prepared by a method that is frowned upon by the medical and pharmaceutical professions (simple dilution of the fluidextract), showed an average activity of 62.6 per cent; or 16.34 per cent stronger than the average for the total fifteen samples, and 24.5 per cent stronger than the ten samples supposedly prepared by the U.S.P. IX method.

The ten samples manufactured according to the method of the U.S.P. IX showed an average activity of but 38.1 per cent.

The results obtained are interpreted as indicating:

(a) A rather general variability of the U.S.P. infusion, all samples examined falling well below the theoretical activity.

(b) A decidedly more active "infusion" when made by dilution of the fluidextract than when prepared by the method of the U.S.P. IX.

(c) The need for an improved method for the preparation of infusion of digitalis. The present official method undoubtedly calls for an insufficient amount of solvent actually employed for extraction, too short a period of infusion, and an insufficiently fine powder.

(d) The need for standardizing the infusion. Many will doubtless look upon this as theoretically desirable, but at the same time impracticable.

(e) The fact that the present infusion of digitalis might be dropped from the U. S. P. without handicapping in any way modern therapy. There is serious doubt whether a standard infusion possesses any advantages over a standard tincture. Laboratory investigations and clinical experiences have shown that the tincture is decidedly more uniform, reliable, and stable than the infusion.

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CHRONIC NEPHRITIS WITH AN UNUSUAL DEGREE OF NITROGEN RETENTION*

By Edward Weiss, M.D., and Vaughn C. Garner, M.D., Philadelphia, Pa.

THE patient, a white male, age 37, a policeman for the past fifteen years, was admitted to the Jefferson Hospital on the service of Prof. H. A. Hare, April 12, 1921, complaining of shortness of breath.

His family history was negative. He had typhoid fever at thirteen, gonorrhea at twenty-two, and influenza at thirty-three (in 1917). From these infections he apparently made a good recovery.

Seven weeks prior to admission he was suddenly seized with dyspnea during the night. This nocturnal dyspnea continued, but up to the time of his admission to the hospital he had been free from discomfort during the day. The only other point of importance in his history was a nocturia of two or three years' standing—he stated that he had to get up one or two times every night to urinate.

Physical examination showed a fairly well nourished, adult, white male with marked pallor. The area of cardiac dullness was somewhat increased to the left; his blood pressure on admission was 220 systolic and 180 diastolic; there was no edema of the extremities. Urinalysis showed a cloud of albumin and a few hyaline and granular casts. Hemoglobin was 56 per cent; Wassermann (blood and spinal fluid) was negative. Eye grounds showed a moderate albuminuric retinitis. The phenolsulphonephthalein test gave only traces of dye elimination in two hours. The two hour test showed a fixation of specific gravity at about 1.011 and a total volume in 24 hours of 1080 c.c.

His general condition grew steadily worse; his nocturnal dyspnea increased in severity. April 19 (seven days after admission) he became drowsy and developed a persistent diarrhea which continued for three days before yielding to treatment. He vomited occasionally and on April 26 a pericardial friction rub was heard. May 1 there was a decided change for the worse and death occurred on May 4.

Of greatest help from a prognostic standpoint were the estimations for nitrogen retention in the blood. These are shown in the accompanying chart. With an initial blood urea-nitrogen figure of 93 mg. this gradually rose and 12 hours before death reached the astounding figure of 304 mg. The creatinine (13 mg.) and nonprotein nitrogen (401 mg.) paralleled this remarkable rise. The asterisk on the chart denotes an estimation of urea-nitrogen (252 mg.) in the spinal fluid. This was found to coincide almost exactly with the blood estimation (246 mg.) done at the same time.

*From the Department of Pathology and Medicine, Jefferson Medical College, Philadelphia, Pa.

Read before the Section on General Medicine of the College of Physicians of Philadelphia, May 30, 1921.

Necropsy was performed the day of death and showed a marked chronic interstitial nephritis, arterial disease and left-sided cardiac hypertrophy with an ulcerative colitis, probably of the so-called uremic type, and serofibrinous pericarditis.

The kidneys weighed but 80 grams each and did not present quite the usual picture of marked chronic nephritis, in that they were not so coarsely granular; advanced scarring was not a feature and there was no increase of pelvic fat; in other words, the kidneys seemed naturally small rather than atrophic. It was felt that, with no etiologic factor discoverable in history or physical examination, such an advanced degree of nephritis in a young man

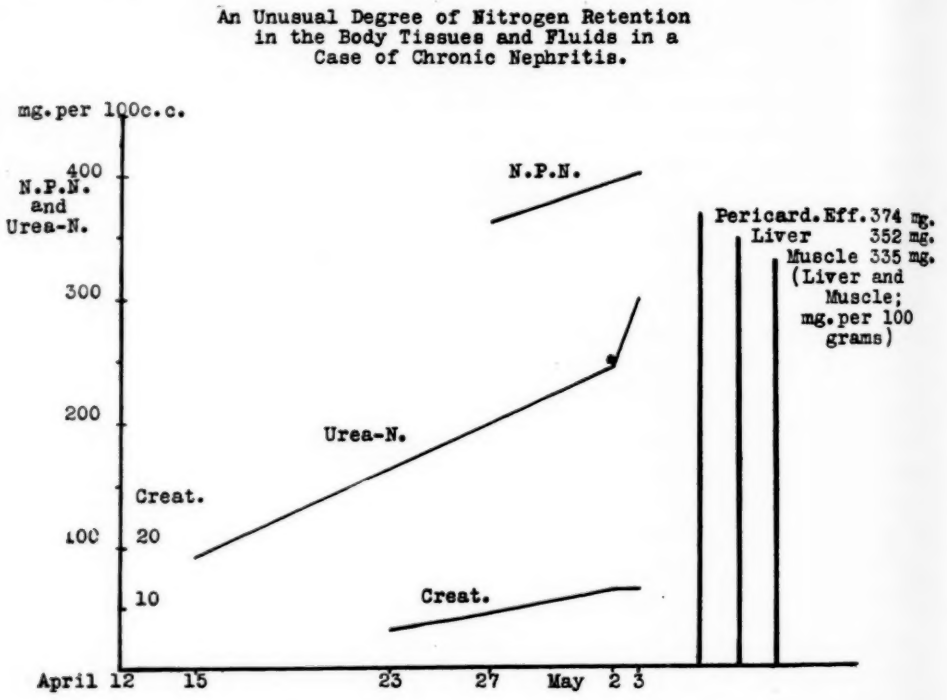

Fig. 1

might point to a congenital basis, in spite of the fact that no definite proof could be offered for this suggestion by the gross appearance of the kidneys.

After death the pericardial effusion, liver and muscle were analyzed for their urea content (denoted by the solid columns on the chart) and it is obvious from the heights of these columns that they approximate closely the blood urea figure (304 mg.) of 12 hours before death—being naturally a little higher because of the time elapsing. This indicates the approximately equal distribution of urea in all the body tissues and fluids—for here we have an estimation of the blood and spinal fluid and later of an inflammatory exudate (pericardial effusion) liver and muscle.

COMMENT

The results of the estimations of nitrogen retention in the various fluids and tissues of this patient agree with the few recorded instances in literature. Marshall and Davis[1] in a study of the distribution of urea analyzed (1) tissue before and after injection of large amounts of urea; (2) tissues of animals whose kidney function had been interfered with—with or without urea injection; and (3) human tissues from necropsies on nephritics. They found the urea content of all organs and tissues approximately uniform and about equal to that of the blood, both in normal conditions and when an abnormally large amount of urea was present. Their experiments show that urea is nontoxic in any moderate or fairly large amount. "Only when introduced in enormous doses—in the neighborhood of 1000 mg. per 100 grams of body weight—does it produce a fatal effect." Therefore, they feel that it is improbable that urea is the only toxic agent in uremia but believe it may take some share in producing the symptoms of the condition. It should be remembered—they add—that urea is equally increased in the central nervous system and at all other points. Our analyses, in the one case at least, bear out this assertion.

Foster[2] in an estimation of the extract-nitrogen of the tissues in chronic nephritis found it to be increased to 50 per cent or more above normal—which he states is always less than 1 gram per 100 grams of dry substance.

Hewlett, Gilbert and Wickett[3] in a noteworthy experiment to determine the toxic effect of urea on normal individuals found that by administering large doses (100-125 grams), symptoms comparable to those encountered in the asthenic types of uremia could be produced. This indicates to them that the high concentration of urea in the body tissues and fluids in nephritis is an explanation in part of the bodily and mental asthenia, but by no means excludes the possibility that other substances may play a rôle in producing the symptoms of this type of uremia, and indeed, the fact that animals die only when extraordinary doses of urea are given suggests that the fatal outcome of asthenic uremia in man may be due to other substances than urea.

Many other observers subscribe to this belief and the remarkable figures for nitrogen retention in states of anuria, without symptoms of uremia, are well known. Only recently Scholl and Foulds[4] recorded a case of prolonged anuria with a urea retention of 528 mg. per 100 c.c.

On the other hand, there is the excellent suggestion of Wells[5] that the reported experimentally determined toxicities with nitrogenous substances have represented only transitory conditions which are entirely dissimilar to the actual conditions in the body. These experiments correspond to the cases of bichloride poisoning, etc., with high nonprotein nitrogen in the blood, in which absence of the uremic symptom complex has been noted and remarked upon. He feels that to study the relation of uremia to retained metabolites, we need observations on their effects when maintained in the organism for long periods at the concentrations occurring in uremics.

SUMMARY

A case is presented of severe chronic nephritis in a young man, which ran a short, fatal course. There was no definite etiologic factor and the thought was suggested that there may have been a congenital basis. The case was marked by an unusual degree of nitrogen retention in the various fluids and tissues of the body. The urea was found rather uniformly distributed and approximately equal to that of the blood (304 mg. per 100 c.c.).

It is felt that if urea is not responsible for the uremic symptoms, it at least serves in many cases as a valuable index to the poisonous substance or substances that are responsible.

We are indebted to Prof. H. A. Hare for permission to publish this case and to Dr. L. A. Hamilton for assistance with laboratory determinations.

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MERCURIC CHLORIDE POISONING WITH RECOVERY A CASE REPORT WITH A NOTE ON THE UREA-CONCENTRATION TEST*

By Elmer H. Funk, M.D., and Edward Weiss, M.D., Philadelphia, Pa.

BICHLORIDE poisoning with recovery, as a result of the modern intensive treatment, is no longer of unusual occurrence.[1] The present case is recorded, however, in order to present the functional studies, which include the urea-concentration test, and in that connection to continue a discussion outlined in a recent case report,[2] of the role of urea-retention in the production of asthenic uremia.

CASE HISTORY

J. T., a white male adult, aged 33, was admitted to the Jefferson Hospital, service of Dr. McCrae, July 21, 1920. On Thursday night July 15th, he had taken four tablets of bichloride of mercury, with suicidal intent. He vomited in less than five minutes and continued to vomit throughout the night. By the next day (Friday) vomiting had ceased but his bowels moved very freely. Saturday and Sunday he was up and about but did not feel able to go to work; Monday, July 19th, he noted that his urine was dark colored and scanty and from then to the time of his admission to the hospital, Wednesday, July 21st, he stated that he had not passed any urine. He applied for admission because of a return of nausea.

Condition on Admission.—A fairly well nourished adult male, mentally clear, who did not appear very sick. The only point of importance in the physical examination was the condition of the mouth; the gums were red and tender and bleeding. He was immediately placed upon the Lambert-Patterson treatment and the subsequent course of the case can best be traced by reference to the chart.

The solid black line, representing the total excretion of urine in c.c. per day, shows 3 days of anuria. Two of these 3 days of anuria occurred before admission to the hospital. On the second day after admission, July 22, he excreted 50 c.c., then 100 c.c. for each of three days; followed by a sharply ascending scale until on August 12, 24 days after admission, he was excreting between 4000 and 5000 c.c. per day and maintained this level until August 27; from then a general decline until upon discharge on September 14, he was excreting a little less than 3000 c.c. a day.

The blood urea-nitrogen began at 140 mg. per 100 c.c. on the 6th day after admission, July 24. This rose to 176 mg. on July 30, and from then a gradual decline in the curve occurred to a normal blood urea-nitrogen (18 mg.) on the day of discharge.

During his days of low total excretion it was impossible to test his phenolsulphonephthalein elimination due to the fact that he secreted no urine during the period of the test. On the seventh day after admission, July 27,

*From the Departments of Medicine and Pathology of the Jefferson Medical College.
Received for publication, July 7, 1921.

his phthalein excretion could be considered a mere trace, and his next estimation on July 31 was no more. From then on the phthalein elimination gradually rose until on the day of discharge it was 45 per cent.

A new method of estimating renal function was used upon this patient.—the Urea-Concentration test of MacLean and DeWesselow,[3] two English observers. This test depends upon the decreased concentrating power of a diseased kidney and is performed in the following manner: fifteen gm. of urea dissolved in 100 c.c. of water, flavored with tincture of orange, are given by mouth after the patient voids. Urine is passed at the end of one hour and at the end of two hours, both specimens being measured and the second, MacLean and Russel,[4] saved for analysis. In those cases showing an excretion of from 350 to 600 c.c. or more in the two hours, any tendency to a low concentration may be put down to excessive fluid, not to kidney

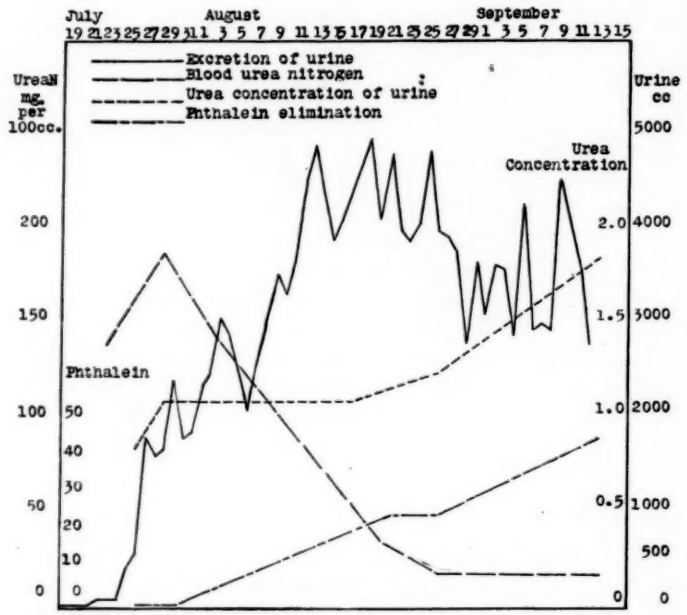

Fig. 1

disease. If the individual can concentrate urea in the second hour specimen to 2 per cent or better, his kidneys are considered efficient; if less than 2 per cent, diseased; and the lower the concentration, the greater the structural damage. It is noted in the present case that a marked parallelism exists between the urea-concentration and the phthalein elimination; the urea-concentration beginning at 0.85 per cent July 27th, and ending at 1.85 per cent September 14th; the latter figure is considered slightly less than normal.

This test has proved a valuable aid in the determination of the efficiency of the kidneys and in a preliminary report it was noted by one of us[5] that there is a close parallelism between the urea-concentration and the phthalein elimination in various grades of chronic nephritis. However, an occasional patient with mild or moderately advanced chronic nephritis, who is capable of normal

phthalein elimination, fails to concentrate urea. This is thought to depend upon one of several advantages which urea possesses as a test substance for kidney function, i.e., the administration of a large dose of urea throws a strain upon the kidneys and thus brings to light latent deficiencies of these organs.

In the present case, though the test gave no more information than the phthalein elimination or the blood urea estimations, it did furninsh some interesting data for comment and speculation as follows:

Lewis and Rivers[6] in a report of a case of bichloride poisoning with complete chemical studies, state in their summary of the case that the retention of waste nitrogen is undoubtedly a factor in the early fatal issue of these cases. Though the height of the nitrogen retention can usually be relied upon as a prognostic sign of importance in these and similar states of anuria, it is doubtful if we have any real proof, any more than we do in true uremia, that the urea or other nitrogen products are the substances actually responsible for the deaths of these patients.

In the present case the 15 gm. of urea administered to the patient on July 27th, in the performance of the urea-concentration test, raised the blood urea nitrogen from 156 mg. to 171 mg. per 100 c.c. in a period of 2½ hours, at which time the maximum blood concentration following urea administration is said to occur.[7] This may have been largely responsible for the high figure, 176 mg., of the next estimation, July 30th, for this test raised the blood urea figure to a similar degree and the same is true of all the subsequent tests. Here then, we had a pathologic accumulation of urea, and similar products, to an unusual degree, augmented by the administration of a large dose of urea on five different occasions. In spite of this procedure, the blood urea curve steadily fell to normal; the patient never showed any untoward symptom which could be attributed to the administration of urea, and on September 15th, 57 days after admission, he was discharged apparently well.*

Though this appears to uphold the contention of many other observers that urea is, for practical purposes, an innocuous substance; and to cast doubt upon the belief that urea, or its allied products may be responsible for certain types of uremia; still, it is well again to call attention to the statement made by Wells[8] that in our efforts to produce uremia experimentally we have failed to maintain urea and its allied substances in the animal body at the constant concentrations over a long period of time at which they occur in many cases of uremia in man.

SUMMARY

A case of bichloride poisoning with recovery following intensive eliminative treatment, is presented.

Functional studies include the urea-concentration test which has been found of more value in the study of chronic nephritis but in the present in-

*The patient returned three months after his discharge from the hospital, Dec. 21, 1920, and at that time presented the following findings: heart, normal in size and function; blood pressure 104/70; eye grounds negative; urine, specific gravity 1023, very faint trace of albumin, an occasional hyaline cast in the centrifuged specimen; blood urea-nitrogen 15 mg. per 100 c.c.; phthalein elimination 60 per cent in two hours; urea-concentration 2.6 per cent.

stance, as an interesting side-light, furnished information as to the comparative innocuousness of administered urea. If the urea-concentration test is to be more generally adopted this observation is of importance.

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LABORATORY METHODS

A NEW MICROCOLORIMETER*

By Victor C. Myers, Ph.D., New York City

MICROCOLORIMETRIC methods which permit the estimation of such blood constituents as hemoglobin, sugar, urea, etc., in the small quantities of blood obtainable from the finger tip or ear lobe have made a strong appeal to many clinical laboratory workers. Although these individuals have conceded that the pain incident to securing blood may often be greater than when larger quantities of blood are properly taken by venipuncture, they have continued to seek micro methods, despite their recognized inferior accuracy. One of the greatest sources of error in these methods has been in the type of colorimeter employed.

The Sahli-Gower hemoglobinometer is probably the most common form of microcolorimeter. Epstein[1] has utilized this instrument in the estimation of blood sugar, while more recently Kuttner[2] has employed the instrument, slightly modified, for a number of micro estimations. Kuttner's instrument differs from the original instrument chiefly in that Helmholtz prisms have been introduced to secure better comparison of the standard and unknown. In this type of instrument the unknown is brought into color harmony with the standard by dilution with water or some diluting fluid. As the writer[3] has previously pointed out, the principle of this method is excellent, and perfectly satisfactory results may be obtained, provided fairly large volumes of fluid are employed (10 c.c. or more) and care is taken in making the dilution. In such small instruments as the Sahli or Kuttner, however, one or two drops, more or less, result in a very considerable error.

With graduated tubes of suitable caliber it is possible to make quite accurate dilutions to such small volumes as 1.5 c.c. How may the color intensity of such small volumes be compared? One does not require a so-called microcolorimeter to make the color comparisons with the micro methods. It is quite possible to make color estimations with 2 c.c. or even 1.5 c.c. of fluid in the Hellige or Bock-Benedict colorimeter, provided that with the latter instrument, the lumen of the cup is nearly the same size as the plunger. Such a cup may also be obtained with the Elei simplified Duboscq. Obviously the various other instruments of the Duboscq pattern can likewise be adapted to this work, although most of these instruments are too expensive to find common clinical use.

Kleiner[4] has recently described a microcolorimeter on the wedge prin-

*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.

ciple, which obviates the dilution of the unknown required for the matching of the standard in the Sahli type of instrument. In the Kleiner instrument the wedge is made by grinding a small bore test tube at a definite angle and fusing it to a ground glass plate. This is used for the standard. When accurately calibrated, good results may be obtained with this instrument, but the difficulty of making two wedges alike is apparent.

In the colorimeter to be described it is believed that this difficulty has been overcome. The instrument is somewhat similar in principle to the Hellige, in which a hollow glass wedge is employed to hold the standard. In the present instrument* a solid wedge of colorless optical glass is fused in the bottom of a test tube of uniform bore. Obviously if the ground portion of the wedge is 100 mm. in length and tapers from the diameter of the tube to 0

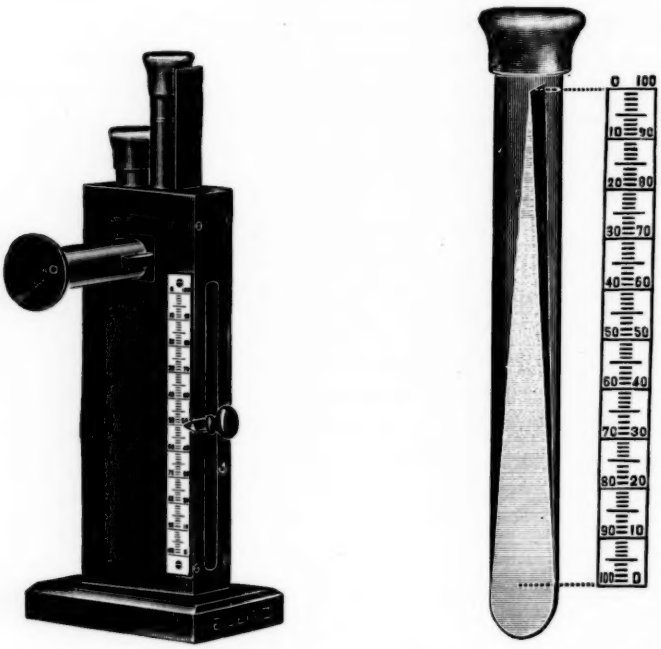

Fig. 1.—Microcolorimeter with accessory wedge to illustrate the principle of the instrument.

mm. and a 100 mm. scale is set on the instrument to correspond with the wedge, (see Fig. 1), a solution ¾ the strength of the standard will read 75, a solution ½ the strength 50, ¼, 25, etc., thus making the calculation very simple, since the readings give the strength of the unknown in per cent of the strength of the standard.

The instrument illustrated above is about 210 mm. in height from bottom to top of tubes, the box without base having a height of 150 mm. The tubes which have been employed have a bore slightly greater than 11 mm., the wedge tube being 150 mm. in length and the tube for the unknown 75 mm. The tube

*The author is indebted to Mr. Walter C. Freisinger, of E. Leitz, for suggesting the use of a solid glass wedge and other help in connection with the construction of the instrument. The instrument is being manufactured by E. Leitz, New York.

for the standard requires 5 c.c. of fluid to cover the top of the wedge, while readings may be made with 1.5 c.c. (and even 1 c.c.) in the tube for the unknown. This tube is graduated at 1.5, 3 and 5 c.c. The 1.0 and 1.5 c.c. dilutions can best be made in a graduated tube of about 6 mm. bore, and the solution then poured in the colorimeter tube.

The instrument is provided with Helmholtz prisms about 4 mm. in thickness, and an eye piece 60 mm. in length containing a lens which increases the size of the field nearly three times (to about 8×14 mm.). The light is allowed to pass through a 25×50 mm. milk glass plate at the back of the instrument, the tubes being separated, and prisms so set, that the light must pass through the center of the tubes. A collar is fitted to the wedge tube so that the wedge must set at a right angle to the light. The tube is provided with a very simple arrangement for moving it up and down so as to bring it into color harmony with the unknown. A pointer indicates the height of the tube on the scale.

The calibration of the wedge tube is very simple. Some stable standard with a good color is taken and dilutions prepared of 25, 37.5, 50, 62.5 and 75 per cent of the standard. Readings are taken and plotted. They should all fall in a straight line. If the wedge is correct for the tube, the readings will be identical with the percentage dilutions. A slight divergence, 1 or 2 points, may be overcome by an adjustment of the scale. If the divergence is more than this, calculation of results may easily be made with the aid of the plane curve which has been plotted.

Standards which are fairly permanent may be left in the wedge tube or fresh standards may be prepared at the time of the test.

The instrument has been employed for micro blood sugar estimations following the micro Benedict technic given by Kleiner[4] and perfectly satisfactory results have been obtained. It should work equally well for hemoglobin, urea, etc. Further discussion of the uses of the instrument will be given at another time.

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SOME TIME-SAVING DEVICES FOR HANDLING PARAFFIN EMBEDDED MATERIAL*

By R. C. Whitman, M.D., Boulder, Colo.

IN THE Department of Pathology of the University of Colorado, all the material for the class in general pathology is embedded in paraffin, except in the few cases where celloidin embedding is especially indicated. The sections are stained and covered for the class and the sections become the permanent property of the student. The labor incident to the preparation of so much material has stimulated us to seek short cuts and labor saving devices to the greatest possible extent. Among these, the following methods, which we have found most useful, are offered here in the hope that they may be of service to others.

1. PREPARATION OF PARAFFIN

We have found that the addition of about 5 per cent white beeswax, (best melted separately, and mixed with the melted paraffin), to the paraffin, as recommended by Grass, in the *Enzyklopadie der mikroskopischen Teknik*, ed. 2, ii, p. 371, renders the paraffin tougher and less brittle.

2. MOUNTING OF SECTIONS

The ribbons of sections are laid on water heated to about 44° in an automatically regulated water-bath. To the water is added white of egg in the proportion of half the white of an egg to six liters of water. A crystal of thymol or carbolic acid, about 1-1000, will prevent the growth of bacteria in the water-bath. When the surface of the water becomes covered by remnants of sections or bubbles, it can be skimmed clean with a sheet of the paper towelling so generally used. Loss by evaporation is made up simply by adding more water. The albumin solution is replaced by fresh solution about once a month.

(The same paper towelling, by the way, we have found to be a cheap and satisfactory filter paper for filtering such things as hematoxylin solutions).

The sections are cut apart with a scalpel, slightly heated in the flame. If the scalpel is too hot, it will melt the sections. If it is not hot enough, the sections will stick to the scalpel. A little practice will enable one to judge the correct temperature very easily. The sections are picked up from the surface of the water on clean slides without the use of any special fixative, the white of egg in the water-bath taking the place of the customary albumin fixative.

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3. DRYING THE SECTIONS

For this we use racks made as follows: Aluminum combs, with teeth far enough apart so that slides will go between the teeth, are fastened side by side two and a quarter inches apart by means of copper tubing such as is used in garages for gas and oil lines. The lengths of copper tubing are placed between the combs, and the combs held in place by stove bolts, which pass through the combs and the copper tubing. Handles made of fairly stiff tinned iron wire are soldered to the copper tubing. The upper end of the handle is bent in a hook to hold a tag. Slides carrying the wet sections are placed in these racks, tagged, and placed in a cupboard heated by electric lamps to dry. As there is no glycerine on the slides, they will dry in an hour or two, and be ready for staining.

4. STAINING

The rack carrying the dry slides is placed in a dish large enough to hold the rack. Formerly, we used the so-called German butter jars, but since these can no longer be obtained, we use dishes made at a local tin shop from copper or tin. The rack of slides is carried as one slide through a series of such dishes holding the necessary xylol, alcohol, water, etc., into the stains, washed, and again passed to the xylol, all as one slide.

5. MOUNTING

The laboratory staff is unanimously agreed that one of the most useful labor saving devices we have worked out consists in the use of an ordinary medicine dropper in the balsam bottle, instead of the customary glass rod. The racks of slides when stained, washed, dehydrated, and cleared are lifted from the xylol and allowed to drain for a moment; the slides laid out on filter paper in groups of ten or a dozen, blotted lightly, covered with balsam by means of the quick-acting dropper, and cover glasses dropped in place. The slides are then picked up and placed in slide boxes. They are then placed in the paraffin oven overnight to dry, and are then put away until needed by the class.

The laboratory staff is agreed that by these methods, cutting sections in paraffin becomes much less time-consuming and laborious than cutting and staining in celloidin.

The most laborious part of the paraffin work remains, of course, the wiping of slides and cover glasses, which, when celloidin is used, is ordinarily done by the student; but a student assistant, working an hour or two a day, keeps us supplied with slides and cover glasses.

The Journal of Laboratory and Clinical Medicine

VOL. VII. JANUARY, 1922 No. 4

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Washington, D. C.

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EDITORIALS

The Nature of Flutter and Fibrillation of the Auricle

THE commonly accepted explanation of the phenomenon of auricular fibrillation is that the auricular tissue is as a whole broken up into lesser or greater numbers of small areas, each of which independently and spontaneously elaborates heterogenetic impulses. The impulses bombarding the ventricular mechanism come at absolutely irregular intervals from scattered and diverse places in the auricles. In fibrillation the ventricles contract absolutely irregularly, without dominant rhythm, and the auricle is described as being in a condition of delirium. In flutter, on the contrary, the auricle beats quite regularly but at a very rapid rate, much more rapidly than does the ventricle.

Thomas Lewis has recently reported experimental work which clarifies greatly our knowledge both of auricular fibrillation and of auricular flutter. The interpretation of these conditions is to be found in the so-called ring experiment, first performed by Mayer in 1908. The experiment has since been repeated on various types of muscle and has been amply corrobrated. The important points are as follows.

If a piece of muscle be so cut as to form a circular band, entirely continuous, but without muscular tissue at the center, electrical stimulation at one point will cause a wave of muscular contraction to spread around the band in each direction, the two waves meeting at a point farthest away from the area of stimulation. Here they cease. The spread is equally rapid in both directions. Fibres which have just contracted remaining temporarily refractory to renewed stimulation, the two waves after meeting do not pass each other and do not continue on around.

Repeated rhythmic stimulation of the muscular band, if timed slowly enough so that the fibres may recover their irritability, will result in repeated rhythmic contraction,—waves beginning at the point of stimulation and spreading in both directions around to the far segment of the circle. It is even possible to start a new wave before the preceding one has completed its circuit, provided the muscle at the point of stimulation has already regained its original irritability. If the frequency of stimulation be increased, the intervals between waves become progressively lessened, until eventually stimuli are applied to muscle which has not completely recovered its responsiveness. Under these circumstances the waves cannot travel with customary freedom in all directions. Occasionally, if one portion of the muscle is particularly unresponsive the wave will be found to travel only in one direction. It will spread in both directions until it meets the unresponsive segment, where one crest will die out, while the other continues on around, in the opposite direction.

If, precisely at this point, with but one wave traveling around the band of muscle tissue, the series of stimulations be discontinued, the wave will continue on around and around, indefinitely. When it reaches a point 180 degrees from the point of starting there will be no wave to meet it and stop its progress, and it will continue on back around the other half of the circle. It will continue on, past the place where the symmetrical wave died out if the fibres have had time to regain their responsiveness. Progressing around the entire ring it comes back to the starting point, to find that this portion of the muscle also has recovered. The one crest is now free to circulate indefinitely, for as it reaches each new segment of the ring it finds that segment again excitable. It proceeds around and around so long as the conditions remain unaltered, and continues its course as a circulating wave without ending.

"Thus it has been shown that, the conditions being rightly controlled, the last stimulus of a series may originate, not a single response of the ring, but an unlimited series of responses. Mines saw the wave continue to circulate for minutes, Mayer saw it continue for many hours; this is what is meant by circus movement; it is constituted by a wave of response, which travels continuously along a re-entrant path of muscle."

In auricular flutter the auricles beat regularly at a rate of from 230 to 350 per minute. The ventricles usually contract at about half the auricular rate,—sometimes a smaller fraction, and are also usually regular, with an occasional jump to the full auricular rate. Lewis has produced flutter in dogs by rhythmic stimulation of the auricle with induction shocks given at a rate of 300 to 600 per minute. The auricle is driven rhythmically and rapidly for

a few seconds and the stimuli are then abruptly withdrawn. In a certain number of such experiments a rapid action of the auricle continues after stimulation has ceased. In some, a typical auricular flutter will thus be produced.

Lewis has mapped out the path taken by the excitation waves both under normal conditions and while flutter is in progress. Normally this wave starts in the sino-auricular node or "pacemaker," situated at the junction between the superior vena cava and the right auricle. From here it spreads simultaneously in all directions, and around both venæ cavæ. The impulses, passing around this circle of veins, meet at the lower point and fuse.

If, on the other hand some point outside the sino-auricular node be rhythmically stimulated, flutter may be produced. If the juncture between the lower vena cava and the auricle, be chosen, the waves will spread up around the ring of vessels, through the sino-auricular node and on around to the starting point. They do not spread equally well in both directions, due probably to an unresponsiveness in certain portions of the muscular ring surrounding the veins. Here we are then dealing with a wave which circulates. It circulates around a natural ring of muscle formed by the orifices of the two venæ cavæ. About 380 circuits are completed in a minute. Flutter consists primarily of a continuous circulating wave. The path may be around the two veins, either clockwise or counter-clockwise. Only one cava may be encircled, or the circus movement may be around other natural orifices such as the mitral valve. The impulse originating in this circus movement spreads rapidly to all parts of the auricular tissue. Thus the movements of the entire auricle are controlled by the circulating wave. "There is the central or mother wave, and there are its centrifugal off-shoots into the remaining tissues." A single wave has thus circulated in certain known cases for as long as seven years.

The circus movement described depends for its maintenance upon three factors; first the length of the path traversed, second the rate at which the wave travels, and third, the duration of the effective refractory period.

"It must be evident that if the wave is to continue circulating a gap must exist between the crest of its advance and the wake of its retreat. As the crest travels it must always find the muscle which it enters in a responsive state. If for any reason the gap becomes closed the wave will proceed no longer and the rapid action of the auricle will cease. The three factors named, and these alone, control the length of the gap. The duration of the refractory period at any given point must always be less than the time spent by the traveling wave in completing its circuit; this circulation time depends on the length of the path and the rate of travel." Lewis has measured these three factors accurately in experimental cases. The gap between the crest of the wave's advance and the wake of its retreat is of extreme importance. Our ability to influence its length will determine our success in treating flutter. If by any means we can close the gap we will bring flutter to an abrupt termination.

The explanation of auricular fibrillation is based upon the same principle but the phenomenon is more complex. When the heart rate is increased, both the duration of the contraction and the refractory period become shortened.

The latter does not shorten proportionately. Consequently the responsive period diminishes when the rate is advanced. At a very high rate the responsive period disappears altogether. When this happens the muscle will not respond to every impulse reaching it and 2:1 block appears. As the rate of beating increases and the period of responsiveness shortens there develops what is spoken of as a partially refractory period. It is a phase of the cycle during which the muscle as a whole may or may not respond to stimulation. There are now three parts to the cycle following contraction; the absolutely refractory phase, the partially refractory period, and finally a short phase in which the muscle is entirely responsive to stimulation. In both fibrillation and flutter the gap between the crest and the wake consists not only of wholly responsive, but also of partially refractory muscle fibres.

"The onmoving crest encounters many fibres which are responsive; it finds some which are still refractory. These refractory fibres form minute barriers to its progress; it wends its way from side to side, passing only where it finds channels open and ready to receive it. It is as though a second prairie fire followed a first, but followed it at a time when the vegetation formerly burnt had not fully returned to its old condition; there would be places where the cinders of the first fire still blackened the earth, and the second flame in passing would creep around the edge of these before it could go forward. These barriers, by deflecting it, render the onrushing wave sinuous in its course, and delay its progress from point to point."

Fibrillation, like flutter, is maintained by a circulating wave or circus movement. Here again there is a single wave but the circuit is completed in a shorter time. The auricular cycles follow each other more rapidly. The path is shorter. This probably is because in auricles predisposed to fibrillation the effective refractory period is shorter than in those predisposed to flutter.

"The difference between fibrillation and flutter is in part one of rate; but that is not the sole difference or even the most important difference. It has been shown to be the rule that in flutter the crest of the circulating wave is constantly passing through tissue in a partially refractory state. The wave as it progresses strikes upon small barriers which render its course finely sinuous. In fibrillation this interference is much exaggerated; the barriers are larger and the crest travels along paths coarsely sinuous. Seemingly the crest has not a straight or simply curved border of advance; it is deeply and irregularly crenated. Its wake is similarly crenated, and the crenations of the one and the other overlap and intertwine. The crest of advance moves through muscle, apparently in a denser state of partial refractoriness than is the case in flutter."

In fibrillation as in flutter the same central path is covered again and again and the wave encircles the same type of central area but it does so in a "staggering" course. It is a road of many obstacles. Progress is therefore more difficult and less rapid, and the return to the starting point occurs after unequal intervals of time. For this reason and because of changes in the actual path pursued, the deflections in the electrocardiogram are of irregular

incidence and form. The paths through the outlying muscle vary. In each cycle the waves pass at least once through the auriculo-ventricular node, but because the path is coarsely sinuous and variable, waves arrive at this node at irregular intervals. The responses of the ventricle are therefore irregular.

Lewis points out that investigation of the refractory period of heart muscle is of extreme importance in the search for therapeutic remedies. Anything which will prolong the refractory phase will aid in closing the gap between the crest and the wake of the circulating wave, and thus will break the tendency to circus movement.

This pioneer work opens new and promising fields in cardiologic study.

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—*W. T. V.*

Intensive Treatment and Neurosyphilis

IN a recent editorial the fact, discovered by Brown and Pearce, that modern antisyphilitic therapeutic measures lower the resistance of the individual to syphilitic reinfection was discussed. It will be remembered that these authors showed that animals were more susceptible to infection after they had received an injection of arsphenamine or neoarsphenamine, than they were if they received no treatment; in other words that, whereas the untreated animals were developing a greater or less immunity to reinfection, the treated ones reacted differently and showed no immunity.

Another aspect of this same problem, based upon extensive clinical rather than experimental observation is taken up by Fraser, whose thesis is that it is the duty of the syphilogist to conserve the antibody supply in order that fewer central nervous lesions be produced, unless he can assure himself that he has prevented a nervous system infection or unless he has killed off every individual spirochete. His argument is based upon the following statement: When the causal organism of syphilis gains entrance, a cell reaction occurs around the organism. A lesion may appear at the site of the inoculation after a certain period of incubation but even before this phenomenon is fully established, the stage of generalization has begun. The chancre is the real protective cell reaction, and the more vigorous this reaction, the longer is the generalization of spirochetes throughout the body delayed. As the so-called secondary stage appears, the reaction of the tissues against the general infection is demonstrated in the rash, or it may be, in a meningeal reaction. The entire destruction of the attacking organism by these reactions seldom takes place, and the organism is permitted to survive and increase. The central nervous system is apt to be invaded at the time of the general systemic invasion, and here, in the absence of a vigorous cell reaction, the spirochete may penetrate into relatively nonvascular structures where it may lead a harmless saprophytic existence for years. While the cell reaction is going on, antibody production is proceeding also, in proof of which is the resistance

to reinfection. The central nervous system, dependent upon the general systemic circulation for its antibody supply, suffers none so long as this supply is available. With this supply cut off the opportunity occurs for growth of the spirochetes and the development of active lesions. The object of treatment then should be, says Fraser, to conserve the antibody supply of the general system in order that it may act, not only generally but also locally in the nervous system. This he says is necessary because the blood circulatory system of the brain and cord is less available for lesions in those organs than in other parts of the body, and that therefore it is possible therapeutically to sterilize the body before the central nervous system is also sterilized. The accomplishment of general sterilization is generally accepted when the blood Wassermann reaction has become negative. At this point treatment is stopped, and thereafter the nervous lesions are left to develop unhindered and with only their locally produced antibodies to influence their development.

As Fraser puts it, "Modern treatment, on account of the tendency to work for a negative Wassermann, plays into the hands of the spirochete. By rapidly sterilizing the general systemic circulation it causes antibody production there to cease. It fails to sterilize the intrathecal system, but successfully cuts off its main source of antibody supply. The result, therefore, is that neurosyphilis occurs earlier and more frequently than was the case in pre-arsenobenzene days." Evidently, if this be true, the physician who wishes to sterilize quickly the patient should do so, and then when the blood Wassermann has become negative, should continue therapy, preferably mercurial, until he can feel assured that the central nervous system too has been rid of its spirochetes. From the laboratory standpoint this would mean when both blood and spinal fluid Wassermanns had become negative. The Army system of antiluetic therapeusis is based upon such a result. But with the laboratory results clinical observation must be combined, if the syphilologist be not "strangled by the tyranny of the Wassermann reaction." Beside the spinal Wassermann, and the spinal fluid lymphocytosis, the gold curve, etc., there are whole series of cases in which there are symptoms which Fraser groups as follows:

The *meningeal group*, in which headache, insomnia, somnambulance, noises in the head, vertigo, slight, severe or complete deafness, optic neuritis, diplopia and paralysis of the recti muscles and even opisthotonus are observed.

The *neurotrophic group* with acneiform and lichenoid syphilides, leucodermia, accompanied by meningeal symptoms and cerebrospinal fluid changes.

The *neurorecurrence group* with affections of individual cranial nerves, inequality of the pupils, sluggish reflexes and cardiovascular changes.

The *psychic group* with its melancholia, irritability, apathy, decreasing memory, moodiness, brutality and even suicidal tendencies.

Largest of all is the *asymptomatic group* in which the spinal fluid usually, but not always, shows suggestive changes.

By systematic study of the clinical phenomena one may judge better than by mere serologic work where a patient stands with respect to his infection, and to a certain extent at least "the method of wholesale arsenobenzene given rigidly and blindly until the Wassermann reaction is negative" can be abandoned. At

the same time a clearer notion of what syphilis may do will be obtained by the physician.

Fraser insists upon the inclusion of *intramine* in any system of treatment of syphilis. This drug he says exerts a protective action upon the nervous tissues. Of it McDonagh says that much nervous syphilis can be prevented by the routine use of intramine which prevents arsenic and mercury from exercising a toxic influence on nerve tissue. It has been used by Ffrench in the treatment of icterus and exfoliative dermatitis occurring during syphilitic treatments.

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—*P. G. W.*

Medical and Health Conditions in Soviet Russia

THERE has recently appeared in the Lancet, London, (April 23, 1921) an article by Dr. Boris Sokolov, Professor of Protozoölogy at the University of Petrograd, in which he depicts the actual state of medical practice and health in Russia. The description is matter-of-fact, but through the lines one may read an infinitude of misery, anguish and suffering.

The Soviet medical organization is based upon rigid centralization, under the control of the Department of Public Sanitation and Hygiene, which directs the entire medical work of the country. The development of a bureaucracy has resulted in an extreme laxity in administration. Directors of the hospitals and clinics are members of the Communist Party and usually are political appointees of little or no ability, but having absolute, dictatorial power.

Scientific work is practically at a standstill. Only a very few medical journals have survived and the laboratories have neither money nor equipment for experimental work. What little research is being carried on has been chiefly in the study of typhus fever.

The Soviet government appears to have at last assumed a tolerant attitude towards the medical profession. Members of this profession are the only "intellectuals" whose work has not been entirely interrupted by the Communist Revolution. The medical men have attempted to maintain throughout a neutral attitude in political matters. As a body they are however, anticommunist. As a result of this neutrality many have been shot by the Reds, and many others by the Whites. Many physicians are still in prison. The doctors have felt it their duty to maintain in so far as possible, the health of the masses, the majority of whom cannot be held responsible for the Revolution or its consequences. Twenty-five per cent of the profession have left Soviet Russia.

Physicians have suffered more from epidemic disease than from persecution or civil war. Between November, 1917, and August, 1920, 47 per cent of the medical men in Petrograd died. Sokolov remarks that it would be difficult to find a single doctor or nurse who had not suffered from two or three attacks of continued fever of some form or other. In the spring of 1920 a gen-

eral improvement in health was noticed. It has been suggested that by this time practically the whole population had been exposed to or infected with the various infectious diseases and that the entire population was practically immune. Within six months' time the percentage of persons suffering from epidemic diseases decreased from thirty per cent to seven per cent.

The economic situation is acute. When the Soviet power was first established equalization of wages was instituted, and experienced doctors earned little more than their assistants. As a result many gave up practice and attempted to earn a living along other lines. Later, however, they were recruited by the Government, and given better salaries. Even now the salaries are preposterously low. Assistants to doctors are paid 4,000 roubles per month. A pound of butter costs 5,000 roubles. The bourgeois clientele of prerevolutionary days can no longer afford the services of the physician. The paying patients now come from the Communist bureaucracy, or are successful speculators. Professor Ov recently charged 100,000 roubles for a small operation performed upon the wife of a leading Communist. This sum is not at all extraordinary. The number of private patients that a physician may have is limited and no doctor may write more than fifty prescriptions for private patients, each month.

The condition in the hospitals is improving, but even now the staff discipline is lax, and the sick are often ill-treated, while delirious patients have been beaten. The doctors do not have full control and can do little to stop this abuse. The central infirmary at Moscow was found in August, 1920, to be in a state of chaos. There was no isolation, and diagnosis was but superficially made. Cases of typhoid and tuberculosis were to be found in the same ward. There was a shortage of medicine and of surgical apparatus throughout Russia. The hospitals in Petrograd and Moscow still had quinine, aspirin and anesthetics, but the reserve stock was nearly depleted. Only a few kilograms of chloroform remained in Petrograd, and all of the morphine had been used up. A salvarsan preparation was being sold, but it was of bad quality. Surgical instruments were running short.

During the last three years there have been 31,789,230 reported cases of infectious disease in Soviet Russia.

Some diseases have disappeared, while new ones have made their appearance. Among the former are obesity and chronic catarrhs. Diseases common among the middle class, brought about by idleness have to a great extent disappeared. A Petrograd physician states that out of 300 patients only nine were found to be suffering from fatty degeneration of the heart and of these, five were well-known Communists and the other four were profiteers. A new illness has recently appeared, due to the eating of badly cooked wheat. The symptoms consist of pain in the right side of the abdomen, dizziness, nausea and distaste for food. Tuberculosis is spreading rapidly and widely. Bad food had brought about a general exhaustion and various forms of anemia. The general ill health is the most striking phenomenon to any one newly arrived in Russia. Professor Ov states that 30 per cent of women and girls are no longer menstruating because of anemia and undernourishment. A large

increase in miscarriages has resulted. Women are terrified at the thought of bearing a child, knowing full well that if born alive the infant will shortly die. The government has abolished open prostitution and has regulated brothels, but there are more prostitutes today than at any time previously.

Sokolov remarks that the greatest danger of recent Russian history is apparent in the younger generation. "Parents are overworked and unable to bring up their children properly; the lack of control is having the most serious results; the actual destruction wrought by the revolution is nothing to the appalling moral degeneracy and criminality among children, which now stands out as the foremost evil of modern Russia. At one hospital, where the number of children suffering from venereal disease used never to be higher than 15 per cent, 60 per cent are now affected as compared with 40 per cent of adults." The children are weak and ailing on account of undernourishment. The new generation is being overcome by eczema, anemia and rickets. The government is now considering nationalization of the children.

Dimitri Mereschkovski has written, in the *Deutsche Allgemeine Zeitung*, "The Bolsheviki preach the destruction of the old civilization in order that a new civilization may be erected in its place. But their words are folly, deception, ignorance; their acts are all that count. We must give them credit that they understand how to destroy. The world has never before seen such destroyers.

"Bolshevism is a reversion to nature; but those who have gone wild in civilized society are powerfully attracted to a state of primitive nature. Europe having reverted to savagery, is drawn toward savage Russia.

"Bolshevism is barbarism, but wearied civilization longs for barbarism as a stifling man longs for air.

"Bolshevism is brutalization. But we must remember that Voltaire said: 'When I read Rousseau, I want to run about in the woods on all fours.' All Europe, when it watches the Bolsheviki, conceives a similar desire to course the forests like the beasts.

"Bolshevism is nakedness; but Europe says, like the dead man in Dostoevski's *Bobok:* 'We, too, will be naked.'

"Bolshevism is the pest, but Europe has already become *A Banquet During the Pest.*

"Bolshevism is the end of the world; but the world wants to end.

"Bolshevism is the suicide of Europe. Tolstoy inspired the act; Lenin completed it." —*W. T. V.*